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FOCUS

Medicinal and theraptic properties of minor fruits - A Review

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ABSTRACT

A large number of minor and wild fruit species have originated in Indian subcontinent. Many of these fruits or their plants parts have been used in folk, Ayurvedic and Unani medicines since time immemorial. Several fruits were introduced in India during colonial period. Most of them adapted to climatic conditions of India but remains minor crops .These fruits also have several medicinal properties and were used by the native people of their respective origin of centres. Some the fruits such as bael, aonla, jamun, tamarind, karonda, wood apple, kokum etc were known for their use in Indian pharmacopeia. The exotic fruits such as sour sop, rambutan, mangosteen, avocado, water apple, durian, passion fruit, carambola etc. have been reported for their different medicinal importance. These fruits contain several chemical ingredients such flavonoids, quinolizidine, alkaloids, tritepenes, stilbenes, tannins, steroids, coumarin, saponins, triterpenoids, glycosides, taraxerone, cryptoxanthin, taraxerol, etc. These exhibited varied biological effects like anti-inflammatory, analgesic, ant diabetic, antipyretic, antioxidant, hypoglycaemic, hepatoprotective, anticancerous. In recent years the global focus is shifting towards the plant based medicines and there is lot of research is being done on these fruits. Thus an attempt has been made in this article to compile the information available in the minor fruits.

***Keywords*:** Antioxidants, medicinal value, minor fruits, theraptic properties

There are more than three thousand of edible fruits species in the world. These were used by human being some times in therein diet. The commercialization of agriculture led emphasis on few edible fruit species. As results of this, more than 75 percent of the global area and production of fruits is comprised by few species such as Banana and plantain, apple, citrus fruits, grapes, etc. (Anomymous, 2019). There are quite a large number of underutilized and wild fruit species, which are being used by the local inhabitants, to meet their dietary requirements **(**Ashrafuzzaman *et al.,* 2021). In fact the underutilized fruits are not only source of food, vitamin and mineral requirements but also the source of livelihood because of their food and curative properties (Das, 2021). Many of these fruits or their plants parts have been used in local medicine. In India many of them are used in Ayurvedic and Unani medicines since time immemorial. Apart from their nutritive and medicinal values quite a few of these underutilized fruits have excellent flavour and very attractive colour. In spite of these quality attributes most have not undergone any conscious phase of domestication and human selection. Their cultivation is very restricted and they grow mainly wild. As far as the native fruits are concerned, several fruit plant species have originated in Indian subcontinent. India is centre of origin of jack fruit, bael, aonla, ber, khejri, jamun, tamarind, mahua, phalsa, Lasoda, karonda, wood apple, pilu, bilimbi, Garcinia, and several other wild fruits (Table 1). Several minor fruits such as Rambutan, mangosteen, longan, avocado, water apple, hog plum, macadamia nut, kiwifruit, longsat, durian, passion fruit, dragon fruit, pulasan, carmbola, etc. were introduced during last few centuries and several are naturalized in Indian conditions (Table 2). Apart from these there more than 100 wild edible fruits native to India which are yet to be domesticated but these are gathered from forest by the rural and tribal people and sold in the rural market (Tripathi *et al.,* 2018).

There is lot of sctter information avaible on the medicinal and therapeutic prpeoerties of minor fruits.Thus an attempt has been made in this artilce to compile these informations in a precise way.

Table 1: Indigenous minor fruits in India

Common Name	Scientific Name	Family	Common Name	Scientific Name	Family
Jack fruit	*Artocarpus heterophyllus*	Moraceae	Tamarind	*Tamarindus indica*	Leguminosae
Aonla	*Emblica officinalis*	Euphorbiaceae	Phalsa	*Grewia subinaequalis*	Malvaceae
Star Gooseberry	*Phyllanthus acidus*	Euphorbiaceae	Bael	*Aegle marmelos*	Rutaceae
Bilimbi	*Averrhoa bilimbi*	Oxalidaceae	Wood apple	*Ferronia limmonia*	Rutaceae
Jamun	*Syzygium cumini*	Myrtaceae	Kokum	*Garcinia indica*	Clusiaceae
Ber	*Ziziphus jujuba*	Rhamnaceae	Yellow mangosteen	*Garcinia xanthochymus*	Clusiaceae
Jherberi	*Ziziphus nummularia*	Myrtaceae	Malabar tamarind	*Garcinia gummigutta*	Clusiaceae
Rose Apple	*Syzygium jambos*	Myrtaceae	Governor's plum	*Flacourtia indica*	Flacourtiaceae
Pommelo	*Citrus grandis*	Rutaceae	Mahua	*Madhuca indica*	Sapotaeceae
Citron	*Citrus medica*	Rutaceae	Pilu	*Salvadora oleoides*	Salvadoraceae
Phog	*Calligonum polygonoides*	Polygonaceae	Ker	*Capparis decidua*	Capparaceae
Sea buckthorn	*Hippophae rhamnoides*	Elaeagnaceae	Indian almond	*Terminalia catappa*	Combretaceae

Table 2: Exotic minor fruits in India

Common Name	Scientific Name	Family	Common Name	Scientific Name	Family
West Indian Cherry	*Malpighia glabra*	Malpighiaceae	Malay Apple	*Syzygium malaccense*	Myrtaceae
Durian	*Durio zibethinus*	Malvaceae	Mangosteen	*Garcinia mangostana*	Clusiaceae
Soursop	*Annona muricata*	Annonaceae	Rambutan	*Nephelium lappaceum*	Sapindaceae
Passion Fruit	*Passiflora edulis*	Passifloraceae	Custard Apple	*Annona squamosa*	Annonaceae

1.0. Phytochemicals in minor fruits

Fruits are rich source of vitamins and minerals. Besides, nutritional importance, these fruits have medicinal and theraptic values. Fruits contain several chemical ingredients such as flavonoids, quinolizidine, alkaloids, tritepenes, stilbenes, tannins, steroids, coumarin, and saponins, quinolizidine, triterpenoids, glycosides, fatty acids, taraxerone, cryptoxanthin, taraxerol, β-carotene, (24R)-24-ethylcholest-5-en-3β-ol glucoside, and β-sitosterol, etc. These exhibited varied biological effects like anti-inflammatory, analgesic, antidiabetic, antipyretic, antioxidant, hypoglycemic, hepatoprotective, and anticancer, dysentery, cholera, wounds, and sores (Doka *et al*., 2014; Ebbo *et al*., 2014; Ibragic and Sofic, 2015; Goyal, 2012). Flavonoids are a large group of natural substances with variable structures present almost in all growing parts of the plants, being reported as the most abundant plant pigment along with chlorophyll and carotenoids, also providing fragrance and taste to fruits, flowers and seeds, which makes them attractants for other organisms. These compounds are also one of the largest groups of secondary metabolites. Besides their relevance in plants, flavonoids are important for human health because of their high pharmacological activities. Recent interest in these substances has been stimulated by the potential health benefits arising from the antioxidant activities of these polyphenolic compounds. Minor fruits are rich source of antioxidants. The antioxidants play an important role in health-promoting biochemical pathways. Oxidative stress, resulting from imbalance among the reactive oxygen species including free radicals and antioxidant defences in living organisms produces oxidative changes to proteins, fatty acids, and DNA molecules in the living cells, which encourage the initiation of ailments, e.g., inflammation, liver cirrhosis and vascular diseases (Aruoma, 1998). Phenolic compounds are the largest group of phytochemicals found in the plants. Phenols, as the major bioactive substances in fruits, play a vital role as antioxidant. Phenolic compounds are good antioxidants found in the flesh of fruits including phenolic acids and flavonoids, whereas flavonoids and lignans are found in the seeds or kernel. Among the phenolic acids, gallic acid is the major component of plant. Each fruit has, at least, a few major phenolic compounds. In addition to fruit, catechin is one of the main flavonoids found in leaves. Since phenolics are potent antioxidants, increased consumption of a mixture of fruits daily should be able to provide an adequate phenolic antioxidant. Among hundreds of types of flavonoids, quercetin is a bioactive flavonoid isolated from the fruit. Besides quercetin, geraniin, quercetin 3-*β*-D-glucopyranoside, kaempferol 3-*β*-D-glucopyranoside, isocorilagin, and kaempferol were detected. To date, only a very limited information on phenolic compounds is available for the scientific community, especially phenolic compounds in the minor fruits such as *Baccaurea*, *Cynometra*, *Garcinia,* durian etc. Monoterpenes, diterpenes, triterpenes, and sesquiterpenes are some of the terpenes. Terpenoid is a vast and diverse class of natural occurring organic chemicals related to terpene. Most of the terpenoids including saponins are possible antioxidants. Besides antioxidant activity, saponins have several health benefits. Among the terpenes and terpenoids, some are volatile compounds found in plants. Geraniol, limonene, linalool, and pinene are some of the volatile components detected in fruit samples. Terpenes, mainly sesquiterpenes, have been identified in the root, bark, flowers, and leaves of plants. Only a few terpenes have been discovered in fruits. Although many studies have been performed on volatile terpenes in essential oils of plants, most of the studies analyzed the other parts of the plant rather than the fruit. From our literature search, a minimum of 20 volatile components including terpenes were found in different parts of the plant. Little information on terpenes and terpenoids content in fruit is available for the scientific community, especially the underutilized and indigenous tropical fruits. The carotenoids are classified as terpenoids. The compounds are found abundantly in yellow to orange- and orange to red-colored fruits. Carotenoids are grouped into carotenes and xanthophylls. In nature, *β*-carotene is the most abundant type of carotene, while lycopene is the primary phytochemical in orange-red colored fruits. Among the xanthophylls, lutein is typically detected in green leafy vegetables. However, some fruits also contain lutein. Among the carotenes, all-

trans *â*-carotene is the most common type of carotenoid found in plant because it is part of the antioxidant defence system at cellular level of a plant. Some green-colored fruits may contain a high amount of carotenoid because the yellow-orange-coloured carotenoid pigments are masked by chlorophylls. The intake of carotenoids from various plant sources is thought to be able to maintain good health. There are several phytochemicals present in minor fruits. Some are unique for particular species and some are present in most of the fruits but their quantity may vary in different plants as well as in their plant parts. Some of the phytochemical presnt in some minor fruits are given in Table 3.

Table 3: Phytoconstituents in various parts of minor fruits

Fruit species	Plant Part	Phytoconstituents
Aonla *(Emblica officinalis)*	Leaf	Gallic acid, chebulic acid, ellagic acid, chebulinic acid, chebulagic acid, amlic acid, alkaloids phyllantine and phyllantidine (Khan, 2009).
	Fruit	Gallic acid 1.32%, tannin, gum 13.75%; albumin 13.08%; crude cellulose 17.08%; mineral matter 4.12% and moisture 3.83%. Amla fruit ash contains chromium, 2.5 ppm; zinc 4 ppm; and copper, 3 ppm (Kumar *et al*., 2012a).
	Stem Bark	Leukodelphinidin, tannin and proanthocy-anidin (Khan, 2009)
	Seed	linolenic acid (8.78%), linoleic (44%). oleic (28.40%), steric (2.15%), palmitic (2.99%) and miristic acid (0.95%) (Khan, 2009).
	Root	Ellagic acid and lupeol (Khan, 2009).
Bael *(Aegle marmelos)*	Leaf	Tannins, Limonene, Aegelin, p- Cymene Phellandrene, Cineole, Skimmianine (Maity *et al.*, 2009); O-(3, 3-dimethylallyl)- halfordinol (Manandhar *et al.*, 1978); Marmelosin (Nandkarni, 1976); Marmesinin, Rutin, β- Sitosterol-D- glucoside, Marmeline(Sharma *et al.*, 1980); Umbelliferone (Arul *et al.*, 2004);Y-Sitsterol, flavones, lupeol, eugenol, citral, Glycoside, O-isopentenyl, Citronellal, Cuminaldehyde phenylethyl cinnamamides (Farooq, 2005)
	Fruit	Alloimperatorin, Imperatorin Scoparone, Scopoletin (Sharma *et al.*, 1980); Auraptene (Kakiuchi *et al.*, 1991); Calcium compounds, Linoleic acid (Maity *et al.*, 2009); Glutamic acid, Glycine, Lysine, Magnesium compounds, Phenylalanine, Proline, Skimmin, Umbelliferone, Xanthotoxol (Barthakur and Arnold, 1989) ; Marmelosine (Badam *et al.*, 2002); Psoralen (Chakthong *et al*., 2012); Luvangetin, Marmelide, Tannin (Farooq, 2005)
	Stem Bark	Fagarine, Marmin (Chatterjee and Mitra, 1949) ;Skimmianine (Maity *et al*., 2009)
	Seed	Anthraquinones (Mishra *et al*., 2009); Linoleic acid, Linolenic acid, Palmitic acid, Stearic acid (Singh and Malik, 2000); Essential oil: D-limonene, A-D-phellandrene, Cineol, Citronellal, Citral, P-cyrnene, Cumin aldehyde (Farooq, 2005)
	Root	Á- Methyl scopoletin, Skimmin, Scopoletin, Timbamine (Shoeb *et al*., 1973); Psoralen, Umbelliferone, Xanthotoxin (Basu and Sen, 1974)
Wood apple *(Ferronia limmonia)*	Fruit	Flavonoids, glycosides, saponins and tannins , tyramine derivatives (Ilango and Chitra, 2010), Flavone glycoside - 5,4-dihydroxy-3-(3-methyl-but-2-enyl) 3,5,6-trimethoxyflavone7-O-b-D-glucopyranoside (Amin *et al*,2017), Citric acid, , alkaloids, coumarins, fatty acids, sterols, umbelliferone, dictamnine, xanthotoxol, scoparone, xanthotoxin, isopimpinellin, isoimperatorin and marmin (Pratima Vijavyargia *et al*, 2014)
	Leaf	Alkaloids - Psoralen, bergapten. Flavones - Orientin, vitexin Saponins Essential oils (Amin *et al*, 2017). Eudesma-4 (Thomas and Ponnammal, 2005), 11-dine (46.3%), carvacrol (29.6%) and 1,5-cyclodecandine (13.4%) , α-Thujene, αPinene, Linalool 0.1, 1,5-Cyclodecandine, Caryophyllene 1.3, cis-Anethole, Elemicin 0.9, Aromadendrene, Germacrene-D, 3,4-Dimethyl cinnamic alcohol, Veratraldehyde, Caryophyllene oxide (Senthil Kumar *et al*, 2010), Stigmasterol,

Contd.

Table 3 Contd.

Fruit species	Plant Part	Phytoconstituents
		orientin, vitedin, saponarin, tannins (Pratima Vijavyargia *et al*, 2014) , Carbohydrate, amino acid, protein, lipid, tannins, alkaloids, steroids (Patil *et al* , 2012)
	Seed	Fixed oil, carbohydrates, proteins and amino acids
	Shell	Psoralene, xanthotoxin, 2, 6-dimethoxybenzoquinone, osthenol (anti-fungal)
		(Ilango and Chitra, 2010), Amino acid, total amino acid (Thomas and Ponnammal,2005)
	Bark	Coumarins - Marmesin, bergapten, psoralen, luvangetin, xanthotoxin, scopoletin, isoimperatorin, osthol and 6,7-dimethoxycoumarin " feronolide and feronone (Pratima Vijavyargia *et al*, 2014), Alkaloids Steroids - Sitosterol and sitosterol-o-beta-d-glucoside. Terpenoids - Lupeol and limonin Flavones - 5, 7-dihydroxy-3', 4'-dimethoxy-6,8-di (3-methylbut-2-enyl) stigmasterol, sitosterol-3-O-β-D-glucopyranoside. The bark of the plant has yielded (-)(2S)-5,3'-dihydroxy-4'-methoxy-6",6"dimethyl chromeno-(7,8,2",3")-flavanone along with several known compounds, five coumarins, a flavanone, a lignan, (antimicrobial) (Ilango and Chitra, 2010),Carbohydrate, amino acid, protein, lipid tannins (Patil *et al* , 2012), Phenols (Thomas and Ponnammal,2005)
	Root	Lactones - Feronia lactone, geranylumbelliferone, frenolin. Coumarin - Aurapten, marmesin, bergapten, xanthotoxin, osthol, xanthyletin, 6-methoxy-7-geranyloxycoumarin, osthenol. Quinolone alkaloid - 1-methyl-4-methoxy-2-quinolone (Amin *et al*, 2017)
Bilimbi *(Averrhoa bilimbi)*	Fruit	Flavonoids, saponins, and triterpenoids (Hock Eng Khoo *et al*, 2016)
Rose apple *(Syzygium jambos)*	Fruit	Phenols, tannins, alkaloids, and flavonoids (Hock Eng Khoo *et al*, 2016)
Malayan apple *(Syzygium malaccense)*	Fruit	Phenolic compounds and terpenes (Hock Eng Khoo *et al*, 2016)
Jherberi *(Ziziphus mauritiana)*	Fruit	Phenolic compounds and saponin (Hock Eng Khoo *et al*, 2016)
Karonda *(Carissa carandus)*	Fruit	L Ascorbic acid, Chlorogenic acid, Ellagic acid, *Piceatannol, Resveratrol. Syringic acid, Vanillic acid, p Coumaric acid, Caffeic acid,* Epicatechin, Rutin, Carissol (Kaunda and Zhang, 2017, Patil *et al*., 2012; Pandya, 2012; Parvin,2018)
	Leaf	Carissic acid, Carissic acid methyl ester, Carissic acid monoacetate, Betulinic acid, Carandinol(Kaunda and Zhang 2017, Patil *et al*., 2012; Pandya, 2012; Parvin,2018)
	Flower	Nerolidol, Farnesol, Camphene, Menthol, p Cymene , α Terpineol, Neryl acetate, Neryl acetate, Geranyl acetate(Kaunda and Zhang 2017, Patil *et al*., 2012; Pandya, 2012; Parvin,2018)

2.0. Minor fruits in folk medicine

A number of species of minor fruits are being used by the people in as suitable food, food supplements and sources of spices and condiments, edible oils, medicine, etc. A number of studies have shown the use of locally available indigenous or traditional fruit speices and their plant parts in medicine by indigenous communities. Some of them are tabulated as Table 4.

3. Medicinal and pharmceatical properties

3.1 Therapeutic values

The therapeutic value of some minor fruits such as aonla (Indian goose berry), Jamun, kokum etc is known since time immortal. Aonla fruit is traditionally known for its medicinal value in India for the treatment of several health complications, suchas diarrhea, dysentery, anemia, jaundice, and

Table 4: Use of minor fruits in folk medicine

Fruits	Use in folk medicine
Bael *(Aegle marmelos)*	Abdominal pain, cholera, night fever, stomach disorder and snake bite. They use specially fruits and roots for treating gastric troubles. Roots of Bael for curing bite of mad dog. leaves use for treatment of heat in abdomen and jaundice, diabetes ,fruit is used as a laxative by different tribes of India (Gupta, 2016), root bark as fish Poison and use the leaf paste as an antivenom against venom of poisonous insects and animals. The powder of fruit and bark is used for the treatment of stomachache and dysentery in eastern Rajasthan (Joshi , 1986)
Bilimbi *(Averrhoa bilimbi)*	Ripe fruits combined with pepper for inducing sweating; pickled bilimbi is smeared all over the body to hasten recovery after a fever; fruit conserves for treatment of coughs, beriberi, and biliousness; fruit syrup for reducing fever and inflammation and to alleviate internal haemorrhoids (Lim, 2012)
Aonla *(Emblica* Officinlis)	Fruit for treating cough and asthma, and several other health complications in Malaysia (Mohamad e*t al*., 2011)
Rose apple *(Syzygium jambos)*	Ripe fruit is used as a tonic for brain and liver and as a diuretic; seeds for treatment of diarrhea, dysentery, and catarrh (Morton, 1987)
Malayan apple *(Syzygium malaccense)*	Fruit decoction as a febrifuge (Morton, 1987)
Jherberi *(Ziziphus mauritiana*)	Ripen fruit for treatment of sore throat and cough; seed for treatment of diarrhea and weakness of stomach [*(*Morton,1987), Marwat *et al*., 2009]
Karonda *(Carissa carandus)*	Roots are used as stomachic, anthelmintic and antiscorbutic; in curing stomach disorders including flatulence and acidity, intestinal worm infestation, diabetic ulcer, scabies, pruritus, pyrexia, urinary disorders, chronic ulcer and biliousness; and even acts as insect repellent (Kumar *et al*, 2013, Trivedi, 2004). Used in the treatment of remittent fever, diarrhoea, earache, mouth and throat soreness, and syphilitic pains, (Kumar *et al.,* 2013 Trivedi, 2004). Unripe fruit used as an appetiser, astringent, antiscorbutic, acidic, stomachic, anthelmintic, laxative and antipyretic, and in curing anorexia, diarrhoea and haematemesis (Kumar *et al*., 2013; Trivedi, 2004). Ripe fruits are used as an appetiser, antiscorbutic and expectorant; in curing anorexia, burning sensation, pruritus and skin disorder; in the treatment of anaemia; as antidote for poisons and carminative; for female libido improvement; in curing worm infestation; as antimicrobial and antifungal; in insanity treatment (Khare 2007; Bisset,1994, Sharma *et al*., 2007).
Jamun *(Syzygium cumini)*	Infusion of fruit or mixture of powdered bark and fruit is used to treat diabetes in North east India(Sharma *et al*., 2001).The juice of ripe fruits is stored for 3 days and then is given orally for gastric problems. The juice obtained from the bark is given orally for the treatment of women with a history of repeated abortion in Lakher and Pawi in North east India. Fruit and stem bark are used in the treatment of diabetes, dysentery, increases appetite and headache in Maharashtra (Jain *et al*., 2005). Decoction of stem bark is taken orally three times a day for 2–3 weeks to treat diabetes (Chhetri *et al*., 2005). Leaves are used in the treatment of diabetes and renal problems by native indians and Quilombolas in North eastern Brazil (de Albuquerque *et al*., 2007). Leaves juice with honey or cow's milk used to treat diabetes. Fresh fruits are taken for stomachache and to treat diabetes by Kani tribals in Southern India (Ayyanar, 2008; Udayan *et al*., 2006). Seeds are taken orally for diabetes in Madagascar (Ratsimamanga, 1998). Dried seeds powder is taken orally thrice a day in the treatment of diabetes in Andhra Pradesh (Nagaraju *et al*. 2006).The juice obtained from the leaves is mixed with milk and taken orally early in the morning, to treat diabetes

Contd.

Table 4 Contd.

	by Siddis in Karnataka (Bhandary *et al*., 1995).The juice of stem bark is mixed with butter milk to treat constipation. Leaves are taken orally for treatment of diabetes in Brazil (Braga *et al*., 2007). The tender leaves are taken orally to treat jaundice in Maharashtra (Natarajan and Paulsen, 2000).
Rambutan *(Nephelium lappaceum)*	Unripe fruit is astringent, stomachic; acts as a vermifuge, febrifuge, and is taken to relieve diarrhea and dysentery. The leaves are poulticed on the temples to alleviate headache. In Malaya, the dried fruit rind is sold in drugstores and employed in local medicine. The astringent bark decoction is a remedy for thrush. A decoction of the roots is taken as a febrifuge.
Kokum *(Garcinia indica)*	Traditionally, kokum is used in herbal medicines to treat diarrhoea, inflammatory ailments, dermatitis, bowel problems, rheumatic pains and to prevent hyper perspiration. Fruits are used as antihelmintic and cardiotonic. Kokum juice from the rind is used against piles, colic problems, dysentery and diarrhoea (Baliga *et al*., 2011). Decoction of fruit rinds are traditionally used against diabetes. Kokum butter is used traditionally to healwounds, fissures in hands and is supposed to restore elasticity of skin and used as a moisturiser (Jeyarani and Reddy, 1999; Padhye *et al*., 2009). Leaves of *G. indica* are used to treat skin ulcers, dyspepsia and hyperplasia.
Malabar Tamarind *(Garcinia gummigutta)*	Treatment of edema, delayed menstruation, ulcers, open sores, hemorrhoids, fever, rheumatism, and also against intestinal parasites (Majeed *et al*., 1994, Semwal *et al*., 2015). The astringent properties of the rind make it an indispensible ingredient in gargles for weak gums, bowel complaints, constipation, diarrhoea and dysentery. The plant is used in veterinary medicine, for mouth diseases in livestock.
Yellow mangosteen *(Garcinia xanthochymus)*	Plant is widely used as a traditional folk medicine for bilious condition, diarrhea, dysentery, anthelmintic, cardiotonic and as a tonic to improve appetite (Whitmore, 1973; Perry, 1980; Baishya *et al*., 2013; Joseph *et al*, 2016). In traditional Chinese Dai medicine, it is used for expelling worms and removing food toxins (Lin *et al*., 2003)
Mangosteen *(Garcinia mangostana)*	Peel and seed in the form of infusions and decoctions used to treat infections of skin, urinary tract, and gastrointestinal, and act as laxative, anti-scorbutic, and anti-fever agent (Ovalle-Magallanes *et al*., 2017). Treatment of diarrhea, abdominal pain, dysentery, suppuration, wound infection, and chronic ulcer (Cui *et al*., 2010; Gorinstein *et al*., 2011; Suksamram *et al*., 2006) and to treat inflammatory and immunological related-diseases, such as acne, food allergies, and arthritis (Wang *et al*., 2017).
Passion fruit *(Passiflora edulis)*	*Passiflora* (or Passion flower) is a folk remedy used for anxiety (Miyasaka *et al*., 2007; Reginatto *et al*., 2006). Several species of *Passiflora* have been employed widely as a folk medicine because of sedative and tranquillizer activities (Barbosa *et al*., 2008). *Passiflora*, an herbal medicine, could be an op-tion for treating anxiety if shown to be effective and safe (Miyasaka *et al*., 2007).
Jack fruit *(Artocarpus heterophyllus)*	The leaves are useful in fever, boils, wounds and skin diseases. The young fruits are acrid, astringent, and carminative. The ripe fruits are sweet, cooling, laxative, aphrodisiac and also used as a brain tonic. The seeds are, diuretic, and constipating.The wood is nervine, antidiabetic, sedative and is useful in convulsions (Hemborn, 1996). The latex is useful in dysopia, ophthalmic disorders and pharyngitis and also used as antibacterial agent (Sato *et al*., 1996). The ash of Jackfruit leaves is used in case of ulcers. The dried latex yields artostenone, convertible to artosterone, and a compound with marked androgenic action. Mixed with vinegar, the latex pomotes healing of abscesses, snakebite and glandular swellings (Vaidya Gogte, 2000). The root is a remedy for skin diseases and asthma. An extract of the root is taken in cases of fever and diarrhoea. The bark is made into poultices. Heated leaves are placed on wounds. The wood has a sedative property and its pith is said to be abortifacient. Latex is used as an anti-inflammatory agent (Gupta and Tandon, 2004).

cough (Chopra *et al.*, 1992). The fruit is also rich in antioxidant. A study on the healing activity of ethanolic extract of aonla fruit has shown some positive results,where the rats were induced with indomethacin (30mg/kg BW, oral intubation) (Bhattacharya *et al.,* 2007). The results showed that the extract (100mg/kg BW) of this fruit had significantly reduced the lipid peroxidation parameters (MDA, carbonyl, total DNA, SOD, and CAT), ulcer index (3.8), and DNA damage induced by indomethacin (85.73% of protection) in rats after seven days of postulcerative treatment compared with the controls. Other than that, the extract of emblic fruit also inhibited the growth of *Staphylococcus aureus, Bacillus subtilis, Salmonella paratyphi, Shigella dysenteriae,* and *Candida albicans* (Ahmad and Beg, 2001). The aqueous extract of aonla has shown the potential as an anticancer agent, where the extract inhibited the growth of human lung carcinoma and (A549) and human hepatocellular carcinoma (HepG2) celllines (Bhattacharya *et al.,* 2007). Moreover, the emblic fruit powder demonstrated a significant chondroprotective effect based on an *in vitro* model of cartilage degradation in explant cultures of articular knee cartilages obtained from osteoarthritis patients (Sumantran *et al.,* 2008). The fruit extract of jamun is reported on the medical properties.The methanolic extracts of jamun leaves were tested for antimicrobial activity, where the extracts inhibited the growth of some Gram-positive and Gram-negative bacteria (Mohanty and Cock, 2010). Besides that, antimicrobial activities of the extracts of bark, leaves, and seeds of rose apple have also been reported by Murugan *et al*. (2011). The leaves of *S. malaccense* (Malay apple) were reported to be useful for preventing inflammation (Cox,1993). Most of the plants from genus *Garcinia* have medicinal effects. In Southeast Asia, only a few studies were reported on the potential medicinal properties of underutilized Garcinia fruits. The fruit extract of *G. hombroniana* inhibited in vitro lipid peroxidation and had antiplateletactivities (Jantan *et al*., 2011). Kapadia and Rao (2011) report antimicrobial effects of Garcinia plants towards bacteria, fungus, and other parasites. The stems and leaves of three Garcinia plants indicate platelet-activating factor antagonist activity (Hemshekhar *et al*., 2011). The main bioactive compound in the leaves that possess antimicrobial effect is reported as garcihombronane (Kapadia and Rao, 2011). Most of the plants from genus *Garcinia* have antihypercholesterolemic effect (Hemshekhar *et al*., 2005). The fruit extract of *G. hombroniana* inhibited in vitro lipid peroxidation and had antiplateletactivities (Jantan *et al*., 2011). Indian jujube is known for its medicinal properties. It is traditionally used for treating abscesses, wounds, anodyne, and tonic, as well as styptic and purifying blood. The leaves of *Z. Mauritiana* were reported to significantly prevent leucopenia and noise induced enhancement of neutrophil function in Guineapigs compared with diazepam, in which the Guinea pigs were subjected to 100Db industrial noise (8–50 kHz) (Vakharia *et al.,* 2014). Antioxidant activities have also been determined for the fruits from two varieties of *Z. mauritiana*, and the I50values of the ethanolic extract of both varieties (Beri and Narikeli) were 72 and 250 ug/mL, respectively (Nimbalkar and Rajurkar, 2009). The ethanolic extracts of *Z. mauritiana* seed were found to induce cancer cells death and significantly reduced tumor volume and tumor cell countin albino mice after 13 days of treatment with the extract (100–800mg/kg BW) (Mishra *et al*., 2011). Besides that, the seed extract exhibited hypoglycemic activity, where administration of the extract (at a concentration of 800mg/kg BW) reduced weight loss and mortality of alloxan-induced diabetic mice (Bhatia and Mishra, 2010). The fruit of bilimbi (tree) has potential health benefits. According to Ambili *et al* (2009), the extracts of bilimbi exhibited the cholesterol-lowering potential in rats. The water extract of bilimbi fruit (0.8mg/kg body weight) improved lipid profile in Triton-induced hypercholesterolemia in rats. The active fraction of the water extract at a dose of 0.3mg/kg BW possessed an optimum antihypercholesterolemic activity. The fruit (125mg/kg BW) and its water extract (50mg/kg BW) also effectively improved the lipid profile of the rats fed with high-fat diet (Ambili *et al*., 2009). Besides that, this fruit is also reported as an active antimicrobial agent. Chloroform and methanolic extracts of bilimbi were reported to have good inhibitory activities on several types of bacteria, such as *Aeromonas hydrophila, Escherichia coli, Klebsiella*

pneumoniae, Saccharomyces cerevisiae, Staphylococcus aureus, Streptococcus agalactiae and *Bacillus subtilis* (Wahab *et al*., 2009). Flavonoids and anthocyanins in dabai fruit (*C. odontophyllum*) are the potent antioxidants. The defatted dabaiextract (5%) was shown to significantly reduce the levels of total cholesterol and low-density lipoprotein-cholesterolin rabbits supplemented with high-cholesterol diet for eight weeks as compared to the control group (Nurulhuda *et al*., 2013). Besides that, rabbits fed a high-cholesterol diet and defatted dabaipulphave a significant increment in high-density lipoproteinlevel (Nurulhuda *et al*., 2012). The severity of atherosclerotic plaques in the high-cholesterol diet rabbit group that supplemented with defatted *dabai* extracts was also reduced compared to the control group. Durian possesses some anti-inflammatory properties. The methanolic extracts of *D. zibethinus* fruit were reported to have anti-inflammatory effects (Leverett *et al*., 2005). The fruit *of Cynometra cauliflora* possesses antiproliferative activity by inhibition of cytotoxic effect to human promyelocytic leukemia HL-60 cells (Tajudin *et al* , 2012).The fruit of *Syzygium jambos* (rose apple) has been traditionally used as an astringent and for brain and liver, as well as digestive problems (Reynertson, 2007). The aqueous fruit extracts of rose apple reduced the *in vitro* glucosidase and amylase inhibitory activities (Das *et al*., 2012). The fruit extracts of *Syzygium samarangense* (samarang apple) were also as effective as antibiotics to inhibit microbial activities (Ratnam and Raju, 2008). Aqueous leaf extact of pasion fruit was more effective in suppressing the TNFα and IL-1β levels than dexamethasone. This may be a source of new therapeutic candidates with a spectrum of activity simi-lar to the current anti-inflammatory steroids such as dexamethasone (Montanher *et al*., 2007).

3.2 Antidiabetic activity

Diabetes has become a common disease around the world. When the body cannot produce sufficient amount of insulin the blood glucose level increases. Antidiabetic aims at reducing the blood glucose level by inducing the production of a higher amount of insulin. Several plant and their parts have anti diabetic properties. Antidiabetic effect of Jamun is well known in Ayurveda. The seed powder of Jamun is effective in controlling high blood sugar levels. The hypoglycaemic effect of different parts of Jamun to control diabetes in preclinical models has been reported. Majority of the preclinical reports have indicated that different parts of Jamun reduced blood sugar levels in rodent models of diabetes and clinical setting. The seed powder extracted in water has shown to reduce blood sugar level in diabetic rabbits (Brahmachari and Augusti, 1961).The use of aqueous seed extract of Jamun at a dose of 1 g/kg body weight in diabetic rats has been reported to produce hypoglycaemic effect in the blood (Kedar and Chakrabarti, 1983). Numerous studies on aqueous seed extract have been found to reduce blood sugar in the diabetic rats (Achrekar *et al*., 1991; Prince *et al.,* 1998). The lyophilized powder of aqueous seed extract has been reported to decrease the blood glucose level in diabetic mice and rats (Vikrant *et al*., 2001; Grover *et al.,* 2001).Similarly, aqueous jamun seed extract consisting of gummy fibres has been highly effective in controlling diabetes in alloxan induced diabetes in rats. The alloxan-induced diabetic rats and rabbits administered with ethanol extract of jamun seeds showed a decline in fasting blood glucose levels in an earlier study (Sharma *et al.,* 2003). The jamun seed kernel extracted in ethanol reduced the blood glucose level in the streptozotocin-induced diabetic rats and also restored the activities of catalase, glutathione peroxidase and superoxide dismutase enzymes accompanied by restoration of glutathione concentration in liver and kidney of diabetic rats (Ravi *et al.,* 2004). Administration of various doses of jamun seed powder into streptozotocin-induced diabetic rats has shown attrition in the fasting glucose level (Sridhar *et al.*, 2005). Likewise, treatment of streptozotocin-induced diabetic rats with 100mg/kg body weight seed kernel ethanol extract reduced the blood sugar level, urea and cholesterol and led to a rise in the glucose tolerance and decreased the glutamate oxaloacetate transaminase and glutamate pyruvate transaminase activities (Ravi *et al.* 2005). Bael (*Aegle marmelos*) has been used as a herbal medicine for the management of diabetes mellitus in Ayurvedic, Unani and Siddha systems of medicine in India (Choudhry *et al*., 2003). Bael extract, when

administered at a dose of 250 mg/kg of body weight, shows better result than glycenamide (antidiabetic drug). This antidiabetic effect may be due to the coumarins present in the fruit which induce the beta cells of islet of Langerhans to produce insulin. Aqueous extract of bael seeds reduces blood glucose level in case of severe diabetic patients (Maity *et al*., 2009; Kamalakkannan and Prince, 2003). Aqueous extract of bael leaves were useful in the long-term management of diabetes due to hypoglycemic and antioxidant effect (Upadhya *et al.,* 2004; Maity *et al.*, 2009). Similarly, anti hyperlipidaemic activity of aqueous extract of bael fruits was demonstrated by Marinzene *et al.* (2005), using the streptozotocin induced diabetic. Sunderam *et al.* (2009) worked on alcoholic extract of bael and jamun in diabetic rats and confirmed their protective activity against laboratory induced cell necrosis. Leaf extract of bael on Alloxane induced diabetes and reported that used extract was enough capable to reduce oxidative stress by scavenging lipid peroxidation and enhancing certain anti oxidant levels which causes lowering of elevated blood glucose level (Kuttan and Sabu, 2004). Rambutan reported to have hypoglyric properties. Geraniin, an ellagitannin, a major bioactive compound isolated from the ethanolic extract of rind act as an anti hyperglycemic agent In addition to its extremely high anti oxidant activity and low pro oxidant capability. Geraniin has the potential to be developed into an anti hyperglycemic agent (Uma *et al*., 2011). Geraniin, one of polyphenol compounds, was used as an index to investigate the optimum condition of extraction from rambutan peel (red and yellow) in Taiwan. The highest total phenolic content found in red rambutan variety was at 1:15 (g/ml) ratio, but no significant difference for yellow rambutan. FRAP ranged in Rambutan rind ranged from 3800.25±86.49 to 4116.5±88.41 (ìmol Fe2+/g dry weight), flavonoid from 6.41±0.48 to 8.57±0.35 (mg Quercetin/g dry weight) and total phenolic recovery from 297.78±4.06 to 358.42±4.63 (mg GAE/g dry weight). Anti diabetic properties of jack fruit is also reported**.** According to a study carried out by Fernando *et al*. (1991), the hot water extract of jackfruit leaves significantly improved glucose tolerance in the normal subjects and the diabetic patients when investigated at oral doses equivalent to 20 g/kg. It also exhibited hemagglutination activity against human and rabbit erythrocytes. Rambutan fruit peel have antidiabetic and antihypercholesterolemic activities. The highest percentage reduction in blood glucose and cholesterol levels are shown of rambutan fruit peels extract with dose 500 mg/kg and the value of percentage reduction were 61.76±4.26% and 60.75±8.26 (Muhtadi *et al*., 2016).The fruit of bilimbi has antidiabetic effect studied using streptozotocin-induced diabetic rats (Tan *et al*., 1996) . The flavonoids, carotenoids, and terpenes could be the potent bioactive compounds in bilimbi fruits that provide the antidiabetic effect. Aqueous leaf extract of graviola to streptozotocin-induced diabetic rats sas found to reduce glucose levels treated diabetic rats along with an elevation in blood insulin (Adewole and Ojewole, 2010). Aqueous fruit rind extract of the kokum exhibited antidiabetic activity in streptozotocin-induced hyperglycemic rats (Kirana and Srinivasan, 2010).

3.3 Antioxidant activity

Normal metabolic activities give rise to free radicals. These free radicals, mainly oxygen free radicals, referred as ROS (Reactive Oxygen Species) causes oxidative stress. ROS are harmful for the body as they damage macromolecules, DNA, proteins and lipids. Antioxidants are compounds that scavenge the free radicals and reduce oxidative stress. Bael fruit has proven to show antioxidant activity. On administration of bael fruit extract of 250 mg/kg of body weight, the activity of ROS scavengers such as glutathione peroxidase, glutathione reductase, superoxide dismutase (SOD) and catalase is shown to increase considerably. Use of above mentioned dose of bael fruit extract shows better results than glibenclamide (36 ìg/kg). The antioxidant activity may be due to presence of flavonoids, alkaloids, sterols, tannins, phlobatannins and flavonoid glycosides (Kamalakkannan and Prince 2003; Singh and Malik, 2000). The rambutan unpigmented fruit flesh, does not contain significant polyphenol content, but its colorful rind displays diverse phenolic acids, such as syringic, coumaric, gallic, caffeic and ellagic acids having antioxidant activity in vitro. Ethanol extract of rambutan fruit peels

contains ethyl gallate (Muhtadi, *et al.*, 2016) which has the strong antioxidant activity. Ethanol extract of rambutan fruit peels are known to have a greater ability as an antioxidant to capture DPPH free radicals than vitamin E (Tamimy, 2006). In rambutan variety**,** ethyl acetate extract of rambutan peels had the highest DPPH scavenging activity with IC50 3.5 ìg/mL, while ethyl acetate extract of binjai rambutan peels had the highest FRAP capacity with EC50 77.1 ìg/mL. N-hexane extract of binjai rambutan peels had the highest total flavonoid (3.46 g QE/100 g), ethyl acetate extract of lebakbulus rambutan peels had the highest phenolic content (40.9 g GAE/100 g) and n-hexane extract of rapiah rambutan peels had the highest carotenoid content (0.61 g BE/100 g). There was a positively high correlation between total phenolic content with their antioxidant activity using DPPH and FRAP assays. In rambutan peel, the extraction of antioxidant compounds, FRAP ranged from 3800.25±86.49 to 4116.5±88.41 (ìmol Fe2+/g D.W), flavonoid from 6.41±0.48 to 8.57±0.35 (mg Quercetin/g D.W), and total phenolic recovery from 297.78±4.06 to 358.42±4.63 (mg GAE/g D.W.) (Azaria and Pi –Jen, 2015). Antioxidant properties in aonla fruit exhibit due to the presence of phenolic compounds (Anila and Vijayalakshmi, 2002; Sabu and Khuttan, 2002; Kumar *et al*., 2006). Total polyphenolic content of different aonla varieties fresh fruits ranged from 70.6 to 159.4 mg GAE/g. Significant difference ($p < 0.05$) was observed in total polyphenol content of different varieties. Similarly, total polyphenol content in aonla powders varied from 90.5 to 385 mg/g, showed significant ($p < 0.05$) varietal difference. Jamun fruit has been reported for strong antioxidant and anti-genotoxic potential. Different parts of this plant are used in herbal formulations. The pulp of the fruit contains diglucoside of five anthocyanidins (Arun *et al.,* 2011; Li *et al.,* 2009). Anthocyanins exhibit anticarcinogenic properties such as induction of cell-cycle arrest and apoptosis, as well as the inhibition of tumor formation and growth in animals (Sabu and Khuttan, 2002). Kokum fruit has high antioxidant properties (Krishnamurthy and Sampathu, 1988; Mishra *et al*., 2006). Choloroform extracts of *G. indica* fruit rinds exhibited excellent antioxidant activities in â-carotene-linoleate and DPPH assays (Tamilselvi *et al.*, 2003). Aqueous extracts of *G.indica* fruits acts as very good antioxidants as evident from their DPPH and lipid peroxidation assays. Aqueous extracts of kokum inhibit ascorbate-Fe2+ induced lipid peroxidation in rat liver mitochondrial fractions (Mishra *et al.*, 2006). Organic acids like citric acid and malic acid from *G. indica* also acts as good antioxidants (Swami *et al*, 2014). *G. indica* bark exudates showed its total phenol and xanthone content as 53.43 g/100g and 32.42 g/100g respectively, revealing it as a potential source of antioxidants (Parthasarathy and Nandakishore, 2016). Palakawong *et al.* (2010) have evaluated the 50% ethanolic extracts of peel, leaves, and bark of mangosteen and found that the highest antiradical activities using DPPH radicals with IC50 of 5.94 ìg/ml, followed by bark 6.46 and leaves 9.44 ìg/ml. Ethanol and n-hexane extract of karonda fruits showed significant antioxidant activities in both extracts compared to ascorbic acid, ASA and tertbutyl- 1-hydroxytoluene (BHT) in 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging with IC of 1.44 and 1.98 ìg/ml of ethanolic extract and n-hexane extract, respectively. Total antioxidant activity and total phenolic content in karonda fruit were comparable to vincristine sulfate, having IC values of 3.43 and 2.66 of ethanolic extract and n-hexane extract, respectively.

3.4 Anticancer activity

Several mnoir fruits have anticarcenogenic properties. Extracts of different plants of sour sop (*Annona muricata)* are well known for their medicinal values. It has been found that the compound present have a variety of anticancer effects including cytotoxicity (Zeng *et al*., 1995; Kim *et al.,* 1998), induction of apoptosis (Eggadi *et al*., 2014), necrosis (Torres *et al*., 2012), and inhibition of proliferation on a variety of cancer cell lines, including breast, prostate, colorectal, lung, leukemia, renal, pancreatic, hepatic, oral, melanoma, cervical and ovarian cancers. The bark, fruit, leaves, root, and seeds, are used as natural medicines in the tropics (Adewole and Ojewole, 2010). The study on methonlic extracts of leaves of jack fruit showed inhibitory effect on various cariogenic bacteria (Sato *et al.,* 1996). The extracts of bael for cytotoxic action using brine shrimp lethality assay; sea urchin eggs assay, and MTT

assay using tumour cell lines. The extract of Aegle marmelos was found to exhibited toxicity on all used assays (Leticia and Costa, 2005). Anticancer effect of hydroalcoholic extract of bael leaves in the animal model of Ehrlich ascites carcinoma and proposed that induction of apoptosis may be due the presence of Skimmianine in extract (Jagetia *et al*., 2005). The anthocyanin i.e., cyanidin-3-glucoside present in kokum fruits decreased the number of non-malignant and malignant skin tumours in the two staged skin carcinogenesis and also caused a dose-dependent inhibitory effect on the migration and invasion of metastatic A549 human lung carcinoma cells (Ding *et al*., 2006). In extract of fruits of karonda was found effective for breast cancer. Free radical scavenging and anticancer activity analysed by DPPH and MTT assays; invasive ductal carcinoma breast cancer protein –aromatase was selected as target which is an oestrogen-synthesising enzyme. IC50 concentration of extract was 86.7308 (µg/ml) found effective against MCF-7 cell lines (Kiruthika *et al*., 2019). Decoction of fruits of passion fruit was found inhibiting activity of gelatinase matrix metalloproteinases (MMP-2 and MMP-9), two metallo-proteases involved in the tumour invasion, metastasis and angiogenesis (Puricelli *et al*., 2003).

3.5 Anti microbial properties

The ethnolic extract of dried fruit pulp of *bael* was found effective against various intestinal pathogens i.e. *Shigella boydii, S. sonnei & S. Flexneri.* This was due to presence of certain phytochemicals such as phenols, tannins and flavonoids (Maheshwari *et al.,* 2009). It was also confirmed by Kaur *et al.* (2009) by getting treat *E. Coli* with bael fruit extract. Citarasu *et al.* (2003) found positive bactericidal effects of bael on certain pathogenic bacteria like *Salmonella typhi, Pseudomonas aeruginosa, Aeromonas hydrophyla* and *Vibrio sp.* The rambutan peel extract exhibited antibacterial activity against five pathogenic bacteria. The most sensitive strain, *Staphylococcus epidermidis*, was inhibited by the methanolic extract (MIC 2.0 mg/mL) (Fidrianny 2015). The methanolic extracts of stem, roots, bark and leaves and seeds of jack fruit exhibit broad spectrum antibacterial properties against various gram positive and negative bacteria (Jagtap and Bapat, 2010; 2013). However the butanol fractions of root bark and fruit have much promising antibacterial activity (Khan *et al.,* 2003). Methanolic extracts of the stem and root, barks, heartwood, leaves, fruits, and seeds of jackfruit have exhibited abroad spectrum of antibacterial activity (Khan *et al.,* 2003). Aqueous and methanol extracts of *Garcinia indica* leaves and fruit rinds showed antibacterial activity against *Salmonella* species (Pasha *et al.*, 2009). Methanol and petroleum ether extracts of both roots and fruits of *Carisaa carandas* compared with standard drug ciprofloxacin (antibacterial activity) and fluconazole (antifungal activity) (Mishra *et al.,* 2009). *G. xanthochymus* showed prominent zones of inhibition against *Vibrio cholera*, *Salmonella typhimurium*, *Shigella flexnerii*, *Streptococcus pyrogens*, *Streptococcus mutans* and *Candida parapsilosis* (Murmu *et al.*, 2016).

3.6. Anti-inflammatory activity

The extract of leaves of bael have anti-inflammatory, antipyretic & analgesic and the extract caused a significant inhibition of the carrageenan-induced paw oedema and cotton-pellet granuloma in rats (Arul *et al.*, 2005). Ghangale (2008) evaluated aqueous extract of bael for anti inflammatory activity by using rat paw oedema model and found anti-inflammatory activity. Shankharananth (2007) found that methanolic extract of leaves of bael @ 200 and 300 mg/ kgBW show significant analgesic activity on acetic acid induced writhing and tail flick test in mice. Rambutan is reported to have many biological activities (Suganthi and Josephine, 2016; Nethaji *et al.*, 2015). The study of anti-inflammatory activities of the aqueous extract of the peel of rambutan *cv.* Malwana special revealed that total phenolic content and the total flavonoid content of AEPR were 463.5±5.2 mg (PGE)/g and 375.0±13.2 mg (QE)/g respectively. The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of AEPR was 3.9-64.5% for concentrations of 10–500 ìg/ml. The hydroxyl radical scavenging activity of AEPR ranged from 10.3-35.0% for concentrations of 1000–1500 ppm. The protection reported for Human Red Blood Cell (HRBC) assay was 17.1-34.8% for concentrations from 35.5-250.0 ppm. Aspirin protection ranged from 32.3-54.5%.

These results show that the AEPR prepared from peelwaste has a potential to be used as an antioxidant and also it possesses anti-inflammatory activity (Uduwela *et al.,* 2019). The anti-inflammatory effects of methanol extract of dried fruits of karonda@400 mg/kg showed significant inhibition of paw volumein carrageenan-induced hind paw oedema in rats (Anupama *et al.,* 2014).

3.7. Antifungal activity

Kokum rind extract showed antifungal effects against *Candida albicans, Penicillium* sp. and *Aspergillus flavus* (Varalakshmi *et al.*, 2010; Tamilselvi *et al.*, 2003). An anti fungal protein was isloted from passion fruit seed. The iso-lated 67-kDa protein, designated as passiflin, exhibited an N-terminal amino acid sequence closely resembling that of bovine beta-lactoglobulin (Lam & Ng, 2009). Patil (2009) reported the antifungal activity of ethanolic extract of the bael leaves. Rana (1997) evaluated anti fungal activity of essential oils isolated from the leaves of bael using spore germination assay. The oil exhibited variable efficacy against different fungal isolates and 100% inhibition of spore germination of all the fungi tested. It was proposed that from bael leaf essential oil may interfere with the Ca-dipicolonic acid metabolism pathway and possibly inhibit the spore formation. Pitre and Srivastava (1987), demonstrate the antifungal activity of ethanolic root extract against *Aspergillus fumiganus and Trichphyton mentagrophytes*. Jackfruit is also known for its antifungal properties. The studies of jackfruit seeds shown to inhibit growth of *Fusarium moniliforme* and *Saccharomyces cerevisiae* (Trinade *et al.,* 2006). It was found that a chitin-binding lectin namedjackin present in the seeds has ability to inhibit the growth of *Fusarium moniliforme* and *Saccharomyces cerevisiae*.

3.8. Hyperlipidemic activity

Hydroxy citric acid is the main active ingredient of most of the species of genus *Garcinia*. It acts in inhibiting the recapture of serotonin, inhibiting acetylcholinesterase, increasing the oxidation of fatty acids, and reducing lipogenesis. The lipid levels were maintained at near normalcy when co-treated with *Garcinia cambogia* extract in dexamethasone administered rats (Mahendran and Devi, 2001). *Garcinia cambogia* showed an antiobesity effect and a significant reduction in the values of triacylglycerol (TAG) of the adipose tissue and liver of the tested groups; however, it significantly increased the TAG pool of the gastrointestinal system. The antiobesity effect and significant reduction of triacylglycerol was also exhibited by aqueous extract of the rind of kokum fruits. Aqueous extract of *Carissa carandas* has been investigated for lipid-lowering activity in egg yolk-induced hyperlipidemic rats. The researchers concluded that the extract exhibited a significant reduction in body weight, cholesterol, triglycerides, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) in model-induced rats. All the results were compared with the standard drug, atorvastatin. Even the histopathological changes in high-cholesterol diet have been reduced significantly with the extract (Sumbul and Ahmed, 2012).

3.9. Hepatoprotective activity

Singanan *et al*. (2007) worked on bael leaf extract on alcohol induced liver injury in albino rats and presented data of excellent hepatoprotective effects. Similarly, Ramnik Singh and Rao (2008) also demontrated that aqueous extarct of bael fruit pulp and seeds are effective in the treatment and prevention of CCl4 induced hepatic toxicity. Methanolic extract of karonda leaves showed hepatoprotective activity using carbon tetrachloride-induced hepatotoxicity in albino rats. The results was as effective as silymarin-treated animals (Bhati *et al*., 2014).

3.10. Antiulcer activity

Oral administration of pyranocoumarin isolated from the seeds of bael showed significant protection against pylorus-ligated and aspirin-induced gastric ulcers in rats and cold restraint stress-induced gastric ulcers in rats and guinea pigs (Goel,1997; Dhuley, 2004) reported that pretreatment of rats with unripe bael fruit extract produce a significant inhibition of absolute ethanol induced gastric mucosal damage. The anti-ulcer activity had examined with different extracts of karonda using petroleum ether, chloroform, alcohol applied on different models using acetic acid-induced chronic gastric ulcer, pylorus ligation and even ethanol-induced acute gastric ulcer, respectively. Oral administration of 500 mg/kg of all extracts would

have enhanced the healing of acetic acid-induced chronic gastric ulcers. Among all the extracts, alcoholic extract showed significant activity in pylorus ligation and stress-induced ulcer. It is concluded that the alcoholic extract of the plant would possess highly potent effect when compared to other extracts (Merai and Jadhav, 2014).

3.12. Anti thyroid activity

Panda and Kar (2006) isolated scopoletin (7-hydroxy-6-methoxy coumarin) from bael leaves and evaluate for its potential to regulate hyperthyroidism. It was observed that scopoletin (at 1.00 mg / kg, p.o. for 7 days) to levo-thyroxine treated animals, decreased serum thyroid hormones level. It was also proved that the scopoletin have superior therapeutic activity than the standard antithyroid drug, propylthiouracil.

3.13. Antinociceptive and anthelmintic activity

Methanol leaf extract of crude drug was investigated for antinociceptive, anthelmintic and cytotoxic activities.Antinociceptive effect of *Carissa carandas* was determinedusing acetic acid-induced writhing assay in Swiss albinomice, whereas in case of anthelmintic activity, the fresh juice of plant leaves was evaluated by recording the time duration of paralysis and the death of *Pheretima posthuma*, earthworms. The cytotoxic activity of methanolleaf extract was analysed using brine shrimp lethality bioassay. Results revealed the potency of the plant that it possesses significant antinociceptive and anthelminticactivity. Methanol leaf extract showed marked cytotoxicproperty as compared with vincristine sulfate used as standard drug (Prveen, 2018).

3.14 Antidiarrhoeal activity

The ethanolic fruit and root extracts of *Carissa carandas* @ 200 and 400 mg/kg BW considerably reduced the total number of wet faeces, compared with standard drug, loperamide (5 mg/kg). Even the ethanol fraction of both plant parts decreased the propulsion of charcoal meal through gastrointestinal tract when compared to atropine sulfate as standard drug. Results revealed the fact that the highest doses of both the plant part extract contained similar effects (Mishra and Sasmal, 2015). In case of chronic diarrhoea and dysentery without fever, half ripe or unripe fruit of bael acts as a remedy. Half ripe fruit is considered best for the purpose but fully ripe fruits or even fruit powder has shown effective results. When the fruit is still unripe, it is cut, dried and ground into powder. The unripe fruit can also be consumed by baking and then consumption with brown sugar or jaggery. After use of fruit, the amount of blood passed in the faecal matter reduces and the faecal matter gets a more solid form (Sharma *et al*., 2006; Patel *et al*., 2012).

Conclusion

It is quite evident from this review that a minor fruits contain a number of phytoconstituents which reveals its uses for various medicinal and therapeutic purposes. The fruits and other plant parts are being used in the folk and tradiotnal medicines by the local inhibitants since long. Some the researches on the phytochemicals present in these speices are being analised and documented. The validation of these pepoerties is also in the progress for the treatment of various disorders in human being such as, diabetes, liver toxicity, fungal infection, microbial infection, inflammation, pyrexia and to relieve pain. Although a part of these fruits have been studied for their medicinal properties, substantial scientific data is still lacking and theresearches are still at a very preliminary stage. Still, very few fruits ahve been studied and most of the much work is required to csriied out in most of these fruits. As the global trends on plant based medicines is radidly growing. The studies need to be performed for the fruits to investigate the mechanism of actions with other therapeutic activities.

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Review Article

Medicinal plants use for home remedies in Sri Lanka: A Review

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ABSTRACT

Sri Lankan home gardens are rich in variety of medicinal plant species. Almost all the parts of the plant have medicinal value hence they are used in traditional Ayurvedic practices. However, leaves, roots, flowers, bark, fruits and rhizome have more medicinal value compared to other plant organs. The present review identifies twenty-five common medicinal plant species that can be easily found in home gardens of Sri Lanka while discussing their applications as home remedies. These plant species could be used to treat stomach pain, diabetes, fever, asthma, constipation, piles, dysentery, menstrual disorders, snakebite and skin diseases due to their biologically active ingredients and medicinal qualities related to antioxidant, antibacterial, anti-inflammatory, antiproliferative, antiviral and anti-cancer.

Keywords: Anti-inflammatory, anti-oxidant activity, home remedies, medicinal plants

INTRODUCTION

Medicinal plants have been recognized for their use in traditional medicine practices since prehistoric times. The potential of plant products in therapeutic and curative ability have been identified back to over five thousand years past, because there is evidence of its use in the treatment of many diseases in Sri Lankan, Chinese, Indian, Egyptian, Greek and old Roman civilizations as well. In Sri Lanka, many medicinal plants have been used as ayurvedic herbs as well as home remedies (Mahesh and Satish, 2008; Nanayakkara and Ekanayake, 2008). Usually, plants synthesize thousands of metabolites (phytochemicals) for their physiological and functional purposes such as defense against diseases, insects, fungi, and many higher animals. These phytochemicals have the potential to establish biological activities which is useful in medicinal purposes. Previous studies showed the potential of medicinal plants to be used as home remedies due to their antioxidant, anti-inflammatory, anti-bacterial, antiviral, antibacterial, antifungal and anthelmintic, properties (Singh, 2015; Samy and Ignacimuthu, 2000; Palombo and Semple, 2001; Kumarasamy *et al*., 2002; Bylka *et al*., 2004). For thousands of years, they have been used to treat and prevent many types of diseases along with epidemics conditions. Some medicinal plants are utilized as pleasant condiments to flavor and colour conserve foods. Further, medicinal plants are used in pharmaceutical, cosmetic and agricultural and food industries (Bamola *et al*., 2018; Arseculeratne *et al*., 1985; Nanayakkara and Ekanayake, 2008; Perera, 2012).

Sri Lanka is one of the biodiversity hotspots in the world with a highest biodiversity per 10,000 square kilometers in Asia (Merritt *et al*., 2019). Traditional medicine has been practiced in Sri Lanka for 3,000 years (Fernando, 1993). At present, four traditional medical systems can be found in Sri Lanka including Ayurveda, Siddha, Unani and Deshiya Chikitsa (Sri Lankan traditional treatment) (Perera, 2012; Fernando, 1993; Kankanamalage *et al*., 2014). The present article reviews medicinal properties and chemical constituents of twenty-five commonly grown pot herbs, medicinal plants, shrubs and climbers that belong to different families which are considered as ingredients for home remedies in Sri Lanka.

HOME REMEDIES

Home remedies can be explained according to medicinal definition as 'simply prepared medication or tonic often of unproven effectiveness administered without prescription or professional supervision'. Home remedies also named as ''folk remedy" can be identified as traditional therapy often utilizing natural products as nutritional supplements or as physical measures. Home remedies come with their effective ness supported by familial, local, or

culturallyaccepted stories or rituals (Mahesh and Satish, 2008; Fernando, 1993). Common home grown medicinal plants such as ginger, turmeric, centella, moringa, sugar cane, ash plantain, ash pumpkin, amla are widely used as remedies (Fernando, 1993; Jayaweera, 1980). Also, known common herbs *Mimosa pudica*, *Gymnema sylvestre*, *Plectranthus zatarhendi, Hemidesmus indicus, Phyllanthus debilis, Tinospora cordifolia* are often used as home remedies (Singh, 2015; Fernando, 1993). Migraine, pimples, prickly heat, worm diseases, toothaches, headaches, fever, cold, muscle cramps like many common diseases are treated using home remedies by Sri Lankans (Fernando, 1993; Bamola *et al.,* 2018; Ediriweera, 2010).

COMMONLY GROWN HOME-GARDEN MEDICINAL PLANTS IN SRI LANKA

1. Ginger (*Zingiber officinale;* Family-Zingiberaceae)

Ginger is a mostly used medicinal plant in Ayurvedic, Chinese, Unani medicines and as home remedies for many ailments such as pain, inflammation, diarrhea, gastrointestinal disorders, stomach aches, vomiting and diarrhea (Srinivasan, 2017). It is commonly grown in many areas of Sri Lanka. Its rhizome is used for the porpose. Many experiments showed that ginger and its active components, 6-gingerol and 6-shogaol, showed anticancer activities against gastro-intestinal cancers (Prasad and Tyagi, 2015; Imo and Za'aku, 2019).The small amount of dry ginger and salt paste is used to increasing appetite. Ginger along with lime and honey is used to treat cough (Fernando, 1993). For diarrhea and colic, a liquid extract of raw ginger, Iriveriya *(Plectranthus zatarhendi)* and Undupiyaliya *(Desmondium triflorum)* mixed with a tablespoon of lime juice and bee honey is used as a remedy. Also, sipping hot ginger tea is a popular and effective sore throat home remedy among many Asians. Ginger and coriander together is used as a remedy for inflammation of the throat and cold. (Malu *et al.,* 2009; Kumar Gupta and Sharma, 2014).

2. Turmeric (*Curcuma longa;* Family-Zingiberaceae)

Turmeric is used as a spice and/or food coloring agent by Sri Lankans. Its rhizome is used for the porpose. Its possible mechanism of action was examined by many researchers. Variety of biological activities including anti-inflammatory, hepatoprotective, antimutagenic and antineoplastic properties due to its phyto-chemical compounds curcuminoids (curcumin and closely related substances) (Tilak, 2004; Hay *et al.,* 2019) were documented may researchers. Turmeric is commonly used to treat skin diseases, fungal infections, pimples and other skin enhancement therapies. For skin diseases, turmeric and neem *(Azadirachta indica)* leaves are boiled together and water extract is used to wash the infected areas. For the fungal infections of the skin, ground raw turmeric and Aththora (*Cassia alanta*) past is applied. A mixture of ground turmeric along with undupiyaliya *(Desmondium triflorum)* is applied for pimples. Also, turmeric's anti-inflammatory, antiseptic and expectorant properties give the ability to fight against cold and chest ailments speedily (Verma *et al.,*2018; Fernando, 1993).

3. Tulsi/Iriweriya (*Plectranthus zatarhendi;* Family- Lamiaceae)

Plectranthus zatarhendi is a plant found in many parts of the country and a common herb in Sri Lankan home gardens. It is a semi-shrubby aromatic perennial (Jayaweera, 1981). Its active compounds are hexatriacontane, lupeol, tannins and oleananes, which are antioxidants and volatile. It has a pleasant aroma when crushed. Its leaves, roots, stems are used. Due to its aromatic qualities, it is used in many remedies for fever, vomiting, diarrhea, excessive thirst and tarantula bites. Dip Iriweriya (*Plectranthus zatarhendi*) roots in water and mix with bee honey and use it to treat diarrhea is a common remedy in Sri Lanka. Further, the oil extracted from *Plectranthus zatarhendi* could be used as hair oil which has aromatic as well as the cooling effect in addition to its ability in hair growth (Fernando, 1993; Lukhoba *et al*., 2006).

4. Red onion (*Allium ascalonicum;* Family-Alliaceae)

The famous folk remedy to keep raw onions in the room when you are sick with cold or fever is very popular for a long time. The bulbs are used. The main active constituents in red onion are phytonutrients like flavonoids, fructo-oligosaccharides, and thiosulfinates and other sulfur

compounds (Slimestad *et al*., 2007) which may facilitate its medicinal properties like anti-inflammatory, detoxifying, antioxidant. Previous studies have shown that the effect of onion on fasting blood sugar and its antioxidant activity on many health benefits (Lukhoba *et al*., 2006). However, red onion is used for many detoxifying remedies for centipede bites, spider bites, some aches, pains etc. Furthermore, red onion is used in many food recipes in Sri Lanka. Even red onion is a popular ingredient in many home remedies for nausea, stomach pain, and other health issues (Jayaweera, 1980; Fernando, 1993; Kumar *et al.,* 2010).

5. Garlic (*Allium sativum;* Family- Alliaceae)

Garlic is quite well-researched herbal remedy which holds a unique position in history, traditionally used for treating infections, heart disease, colds, diabetes and many other disorders. The bulbs are used. Clinically, garlic has been proven for lowering blood pressure, cholesterol, glucose concentrations (Tsai *et al.*, 2012; Imo and Za'aku, 2019). *Allium sativum* is generally attributed to its rich content of sulfur-containing compounds, alliin, g-glutamyl cysteine, and their derivatives (Tsai *et al.*, 2012). It is used to flavor foods as well as treating swellings, detoxifying and reduce cholesterol levels. Garlic is grinding together with curry leaves (*Murraya koenigii*) and Goraka *(Garcinia zeylanica)* until it becomes a paste and adding it to the meal is a commonly used home remedy for cholesterol among Sri Lankans. Garlic is also used as a gastric stimulant agent that aids the digestion and absorption of food. With mustard, garlic is used to treat paralytic and rheumatic affections. *Allium sativum* bulbs are even recommended for high blood pressure. Among many common remedies consuming 5-6 garlic cloves with boiled milk, turmeric and jiggery is known to be a treatment for long-term cold and cough. In addition to that, applying garlic with butter can heal wounds and consuming garlic with sugar syrup helps to treat cough and provides effective relief for cough, nasal congestion and sore throat. (Fernando, 1993; Jayaweera, 1980; Imo and Za'aku, 2019).

6. Curry leaf tree (*Murraya koenigii;* Family- Rutaceae)

Curry leaf is a popular medicinal plant easily found in Sri Lankan home gardens used for many home remedies. The leaves, roots, bark, stalks and flowers all parts have medicinal values (Fernando, 1993; Jayaweera, 1982). Its active phytochemicals are oxygenated monoterpenes which have antioxidant and anti-inflammatory properties. All parts of the plant are used to de-poison snake venom along with some other herbs. Especially the bark and the roots are used as a stimulant by the physicians externally to cure eruptions and the bites of poisonous animals (Singh, 2014). It is used as a treatment for nausea, coughs and fever (Jayaweera, 1982). Curry leaves (*Murraya koenigii*) with Goraka (*Garcinia zeylanica*) paste is used to reduce blood cholesterol (Fernando, 1993; Jayaweera, 1982). Further more, it is used to treat for diarrhea, dysentery, indigestion, peptic ulcers, diabetes and weight loss. *M. koenigii* leaves are used to flavor curries. Also, due to its presence of iron content in leaves, they have found to be solving some problems of anemia. Even curry leaves are used in fresh, dry, paste, or oil form in skin and hair care remedies. *Murraya koenigii* oil is a very popular hair growth-promoting oil among Sri Lankans (Jayaweera, 1982; Singh *et al*., 2014)

7. Ballon plant (*Cardiospernum halicacabum;* Family- Spindaceae)

Cardiospernum halicacabum is an annual herb that can be found as plenty of wiry, smooth, climbers in Sri Lankan home gardens. It is intensively used by the Sri Lankans to make herbal porridge (*Kola kanda*). It shows good results on allay pains, abnormal suppression of menses and other fertility problems of humans. Leaves, roots, fruits are used. The leaves are used as a poultice for skin eruptions. A paste of *Cardiospernum halicacabum* leaves can be used as a dressing for wounds and sores (Fernando, 1993; Jayaweera, 1982). Also, it is used to treat dysentery, rheumatoid arthritis, back pain and hernia and even has been used in traditional medicine for nervous diseases (Jayaweera, 1982).

8. Castor (*Ricinus communis;* Family- Euphorbiaceae)

Leaves, roots, bark, seeds are used for various purposes.. The plant is 3.5-13.5m tall with hollow stems. Seeds are used to extract oil which contains a higher proportion of fixed oil with active components of ricin and an alkaloid ricinine. This chemical ricinine is found in leaves and stems

appear to be non-toxic though the seed is poisonous. Castor is used in many traditional medicinal preparations (Ahmed and Urooj, 2010; Jena and Gupta, 2012). Leaves are used externally for headaches and stomachaches as a boil and rheumatism. The paste of castor roots are applied for toothaches. The root bark is found to be purgative and used as a remedy for skin diseases, burns and sores. A poultice of *R. communis* leaves applies externally to women breast to increase the secretion of milk. The bark is used for dressing sores (Jayaweera, 1980).

9. Aloe (*Aloe vera;* Family – Asphodelaceae)

Aloe vera is a bushy herb with short, thick and fleshy leaves, remedy for skin burns, sunburns and pimples. Leaves/ inner gel are used. Applying Aloe gel on dehydrated skin is commonly practiced. Raw Aloe gel is used to treat gastritis and abdominal pains. *Aloe vera* is used to produce many cosmetic products (Reynolds, 2004; Bamola, 2018). Its active compounds are Vitamin A, C, E, Carotenoids which provide antioxidant, antibacterial, anti-inflammatory effect. Anti-inflammatory and wound healing characters of *Aloe vera* have been scientifically tested (Udupa *et al*., 1994; Davis and Maro, 1989). Aloe is used for coughs, constipation, asthma and nervous diseases in traditional medicine. The fresh gel of the leaves has cathartic and cooling ability and use for various eye diseases. To reduce swellings and promoting granulation in ulcers, the dried Aloe juice along with lime is applied as a remedy. It is mixed with milk and given for dysentery and pains in the kidney. *Aloe vera* gel also applied as a remedy for preventing hair loss and cure baldness, to avoid dryness in the skin as a natural moisturizer (Debjit, 2019; Jayaweera, 1981; Reynolds, 2004; Bamola, 2018).

10. Indian sarsaparilla (*Hemidesmus indicus;* Family- Peripolocaceae)

Indian sarsaparilla is a twining slender prostrate or semi-erect shrub commonly found in home gardens. The whole plant is used for medicinal purposes and active ingredients are alkaloids and glycosides, which have anti-inflammatory properties. The root is used to treat a wide variety of illnesses. This root of the herb can treat patients with chronic skin disease and other conditions such as cough, genitourinary disease, and rheumatism. *Hemidesmus indicus* has the potential to increase appetite, reduce body heat, resolve urine problems etc. This herb is so far identified as a remedy for face skincare (Das and Sigh Bisht, 2013). *Hemidesmus indicus* whole herb is boiled with water and washing out the skin is practiced (Fernando, 1993). This herb is also used to make herbal drinks, tonics and congees as well. *Hemidesmus indicus* is an ingredient for preparations for edema, skin rashes, coughs, asthma and piles (Jayaweera, 1982).

11. Pepper (*Piper nigrum,* Family -Piaparaceae)

Pepper is a climbing perennial commonly found in Sri Lankan home gardens mainly used as a spice. Its active phytochemical is piperine. However, it is used for many remedies including treating dry cough, wheeze and sneezing. Its leaves, root and seeds are used for the purposes. People use black pepper for arthritis, asthma, upset stomach, bronchitis, bacterial infections cause diarrhea / cholera, colic, depression, gas/bloating, headache, menstrual pain, stuffy nose, sinus infection, dizziness, discolored skin (vitiligo), weight loss and cancer conditions (Takooree *et al.,* 2019; Fernando, 1993). Pepper is an ingredient in many traditional medicines. For cough, pepper and sour orange (*Citrus aurantinum*) extract or pepper powder along with sugar and bee honey is used (Fernando, 1993) Furthermore, for many remedies for colds, asthma, worm infections and fever includes pepper can be found as a valuable ingredient (Jayaweera, 1982; Fernando, 1993).

12. Sour orange (*Citrus aurantinum;* Family- Rutaceae)

Citrus aurantinum is an under cultivated tree in Sri Lanka. A tree about 10 m tall bears sour juicy fruits. Fruits and leaves are used for the purposes. It is a well-known remedy for acidity and bloating drinks. Extracted juice of sour orange fruit together with sugar or bee honey is used for cough and cold (Suryawanshi, 2011; Fernando, 1993). *Citrus aurantinum* is a popular treatment for inflamed glands and tonsils, chronic cough and scorbutic conditions. Furthermore, the fruit of sour orange is used in various herbal medicines as a stimulant and appetite suppressant. Even sour orange has found effective for various diseases such as aid in digestion and relieves cardiovascular health, anti-cancer, treatment for strokes etc. (Jayaweera,1982; Fernando, 1982; Suryawanshi, 2011). Many health

professionals recommend dried sour orange peel (1-2g) simmered for 10 to 15 min in a cup of water daily as detoxifying drink and appetizer (Suryawanshi, 2011).

13. Stone breaker /Pita wakka (*Phyllanthus debilis;* Family- Euphorbiaceae)

Phyllanthus debilis is an annual herb used to treat swellings, wounds, coughs, skin rashes, asthma, gonorrhea, coughs, dysentery, diarrhea, fever, ringworm, jaundice, scabies, sores, bruises, tuberculosis ulcers and liver diseases (Jayaweera, 1980). Drinking finely ground plant mixed with pure fresh cow milk is a common remedy for urine problems (Fernando, 1993). This herb has anti-inflammatory properties and leaf juice taken orally for many remedies. Even the *Phyllanthus debilis* herb has been shown to possess maximum antioxidant activity compared to some other species in the genus Phyllanthus (Sarin, 2014; Jayaweera, 1980). Roots and leaves are used for the purposes.

14. Drumstick plant (*Moringa oleifare;* Family- Euphorbiaceae)

Seeds, roots, bark and leaves are used for various purposes. It is a tall tree with pinnate leaves which has long pods 18-45 cm long (Jayaweera, 1980; Gandji *et al*., 2018). Pods are cooked in coconut milk sauce while leaves are added to some other curries and bark is added to pickles. Moringa leaves are rich in nutrition. Hence it could be used to fulfill the nutrient requirement of people who are suffering from deficiencies. *Moringa oleifera* reduce in blood sugar and cholesterol level. Its active ingredients are alkaloids and glycosides. Moringa has antioxidant, antifungal, antiviral, antidepressant and anti-inflammatory effects and can protects against arsenic toxicity. Bark, seeds, roots and resin are used in medicinal preparations for piles, skin diseases and worm diseases. It helps to increase appetite and the function of the kidneys and heart, good for swelling, parasitic diseases and matures tumors (Liyanaratne, 2003; Coppin *et al*., 2013; Jayaweera, 1980).

15. Sensitive plant / Sleeping grass (*Mimosa pudica;* Family- Mimosaceae)

The whole plant of *Mimosa pudica* used in folk medicines (Fernando, 1993; Dassanayake and Fosberg, 1980) due to its phytochemical mimosine. It contains the toxic alkaloid, mimosine, which has been found to have antiproliferative and apoptotic effects (Bamola, 2018). It has been used for treating piles, bleeding ulcers, bleeding wounds, swellings and fistula. Some herbal doctors recommend *Mimosa pudica* for bronchitis. All five parts of the plant; leaves, flowers, stems, roots and fruits are used as medicines in traditional health care methods. This sensitive plant is commonly used for bleeding disorders like menorrhagia, dysentery with blood, mucus and piles and used as an ingredient in many remedies that can cure skin diseases and poisons (Jayaweera, 1982; Dassanayake and Fosberg, 1980).

16. Heart leaved mooseed (*Tinospora cordifolia;* Family- Cucurbitaceae)

It is a comparatively big climber native to Sri Lanka. It has been used for treating fevers, skin diseases, chronic diarrhea and anemia (Jayaweera, 1980). *Tinospora cordifolia* is used as a remedy to enhance digestion. Stem and roots are used for the purposes. An infusion of its stems is used as an alternative tonic for blood purifying purposes. *Tinospora cordifolia* could be used to reduce diabetes. A drink prepared with bee honey and dried powder of thippili (*Piper longum*) fruit is popular among Sri Lankans (Fernando, 1993). Antiviral infections, anticancer, ant diabetes, the antiseptic activity of *Tinospora cordifolia* are due to presence of alkaloids like Berberine and Choline (Mittal *et al*., 2014), Diterpenoid Lactones, Steroids and Sesquiterpenoid (Mittal *et al*., 2014; Modi *et al*., 2020; George *et al*., 2016; Jayaweera, 1980).

17. Centella (*Centella asiatica*; Family- Apiaceae)

A very common bush or creeping type plant found in Sri Lankan home gardens and often consume as a fresh salad. The whole plant is used for various purposes. *Centella asiatica* is a type of leafy plant traditionally used in Asian cuisines. It has a long history of use in both traditional Chinese medicine and Ayurvedic medicine. It is a perennial plant indigenous to the tropical wetlands of Southeast Asia, where it is commonly used as a herbal juice, tea, or green leafy vegetable (Dassanayake and Fosberg, 1980). *Centella asiatica* is used for infections, for the prevention of Alzheimer's disease and blood clots. Further, it is well known for treating or preventing anxiety, asthma, depression, diabetes, diarrhea, fatigue,

indigestion and stomach ulcers (Fernando, 1993; Jayaweera, 1982; Dassanayake and Fosberg, 1980).

18. Shoe flower (*Hibiscus rosa-sinensis;* Family- Malvaceae)

A large flowering shrub with simple alternative leaves bears flowers throughout the year. Its leaves, stems and roots are used for various purposes. Leaves and young flower buds are used as a poultice on boils and swellings. The root is given for fever, cough and venereal diseases. Flowers can be used as poultice to enhance hair moisture and reduce hair damage (Jayaweera, 1982). Remedy for skincare, for example, an extract from the flowers of *Hibiscus rosa-sinensis* has been shown to function as an anti-solar agent by absorbing ultraviolet radiation and for hair care treatments (Nevade *et al*., 2011; Bamola, 2018; Al-Snafi, 2018; Jayaweera, 1982).

19. Morning mallow/Gas bavila (*Sida acuta*; Family- Malvaceae)

Sida acuta is a branched shrub distributed all over the country which flowers all year around. The leaves, stems and roots of *Sida acuta* have been used for traditional medicine. This plant contains alkaloids and asparagine. Roots and leaves are decoction and given for hemorrhoids, fevers, impotency and rheumatism. Crushed leaves with gingerly oil are applied for boils. The juice of roots and leaves are used to remove intestinal worms. (Mahmood *et al*., 2010; Jayaweera, 1982). In addition to that, the root is used as a treatment for bladder irritability and mild cases of fever (Jayaweera, 1982). The pharmacological properties of *Sida acuta* include antimicrobial, antioxidant, anti-plasmodial, cytotoxic activities may be the reason behind these usages and many medicinal values (Karou *et al.*, 2007; Jayaweera, 1982).

20. Lasia (*Lasia spinosa;* Family-Araceae)

Lasia spinosa is a stout stemmed marshy plant growing up to 4 m in height with a thick spiny creeping stem. Stems and young leaves are edible. Leaves are simple and long-petioled 15-45 cm long. Frequently, it is cultivated in marshy areas of home gardens in Sri Lanka. The leaves, stems and roots are used as a common remedy for piles, dyspepsia, stimulating liver functions (Jayaweera, 1980; Fernando, 1993). It is well-known remedy for constipation. Acongee made out with stem is a popular drank to get rid of constipation (Liyanaratne, 2003; Tsai *et al.*, 2012; Jayaweera, 1980).

21. Pomegranate (*Punica granatum;* Family-punicaceae)

Pomegranate can be considered as a large shrub or small tree with 3-5 m tall and slender angular branches. Its flowers, leaves, fruits, root are bark are used for various porposes. The biological properties of pomegranate extracts (antimicrobial, antioxidant, anticancer, anti-inflammatory, among other properties) have been tested and used in therapeutics, such as in the prevention of infection, inflammation, cancer, among other applications (Miguel *et al*., 2010; Lansky *et al*., 2000). It is a remedy for eye infections, worm diseases, asthma and fevers. For eye infections, leaves are boiled in water and washed eyes thoroughly. The root bark is specifically useful for tapeworm and tuberculosis diseases in children. Fruit contains inverted sugar, enzymes, citric acid and malic acids as well as rich in vitamin C and Iron. The fruit is used to treat diarrhea, dysentery and given to cancer patients. Furthermore, the bark of stems is used as a medicine due to its anthelmintic properties (Fernando, 1993; Jayaweera, 1982).

22. Long Pepper (*Piper longum;* Family-Piperaceae)

It is a perennial herb or climber with cylindrical spikes with small blackish-green fruits. Immature spikes, roots have medicinal properties are used. Chronic bronchitis, fever, cough, piles can be treated using *Piper longum* as an ingredient in many remedies. (Jayaweera, 1982). Extract of *Piper longum* fruits has been tested for its immunomodulatory, anti-inflammatory and anti-asthmatic properties. Fruit of *Piper longum* contains alkaloids of piperine, volatile oils and resins (Vinay *et al.,* 2012).The fruit is also used with other ingredients to treat enlargements of the spleen and some other abdominal viscera. *Piper longum* fruit is a stimulant for the urethra and rectum and it enhances gastric secretion and improves appetite. Therefore, many remedies to increase appetite include *Piper longum* as an ingredient. The roots are used due to their laxative, carminative and expectorant properties in Sri Lankan traditional medicine (Ali *et al*., 2007; Khushbu *et al*., 2011; Jayaweera, 1980).

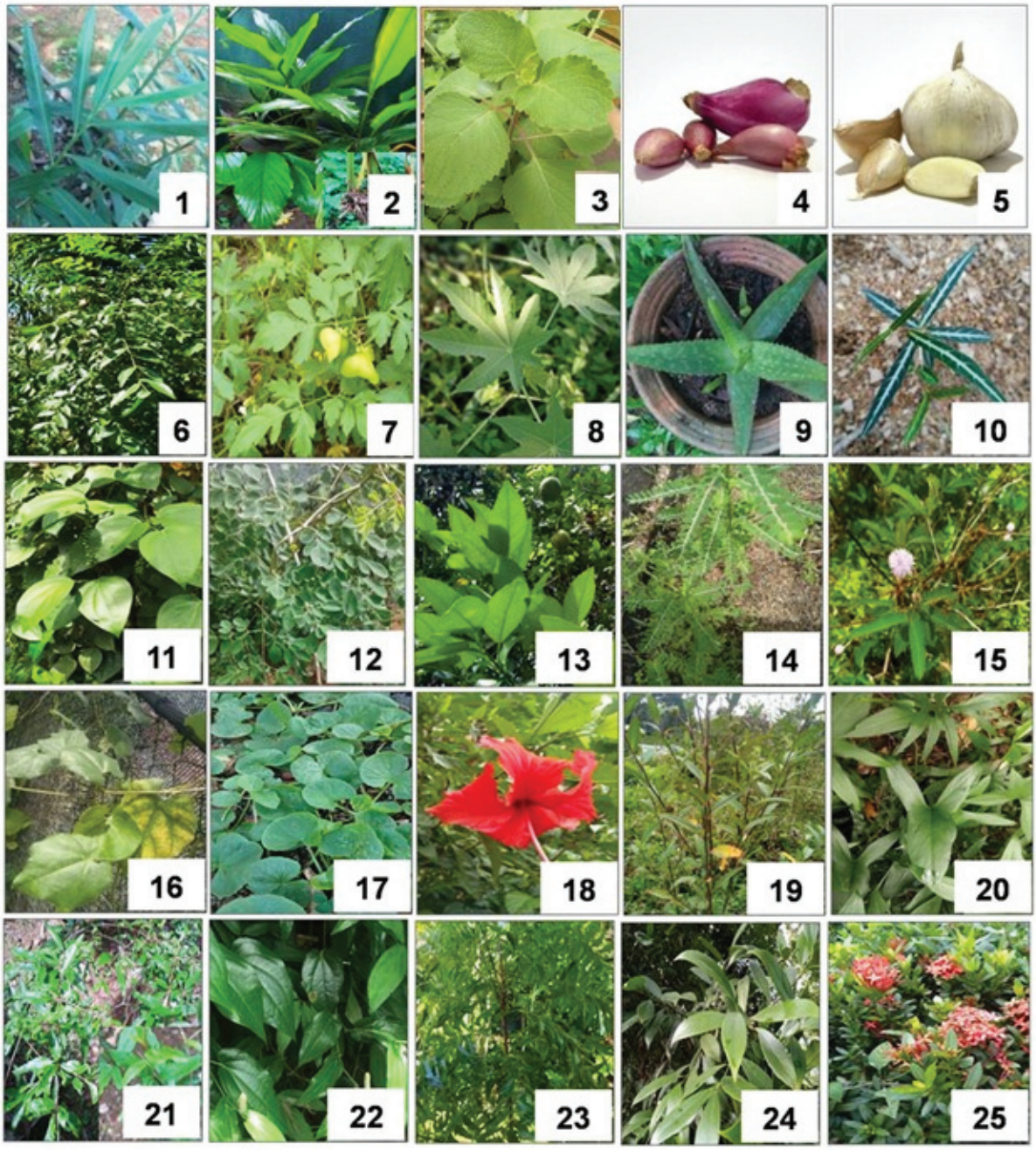

Fig. 1: 1- *Zingiber officinale*; 2- *Curcuma longa;* 3-*Plectranthus zatarhendi;*4- *Allium ascalonicum*; 5- *Allium sativum;* 6- *Murraya koenigii* 7-*Cardiospernum halicacabum;* 8- *Ricinus communis;* 9- *Aloe vera;* 10-*Hemidesmus indicus;* 11- *Piper nigrum;* 12- *Citrus aurantinum;* 13-*Phyllanthus debilis;* 14- *Moringa oleifare;* 15- *Mimosa pudica;* 16-*Tinospora cordifolia*; 17-*Centella asiatica*; 18- *Hibiscus rosa-sinensis;* 19-*Sida acuta;* 20-*Lasia spinosa;* 21-*Punica granatum;* 22- *Piper longum;* 23-*Azardirachta indica;* 24- *Garcinia zeylanica;* 25-*Ixora coccinea*

23. Neem/Margosa (*Azardirachta indica;* Family- Meliaceae)

Neem is a large perennial tree with spreading branches having natural antiseptic characteristics (Jayaweera, 1982). Its active phytochemicals are nimbin, nimbinin, nimbidin and nimbidiol which provide aseptic, antibacterial, anti-microbial effect. Leaves, bark and seeds are used for various purposes. It is an ingredient in many remedies for fever, skin ailments, wounds, coughs and some worm diseases (Jayaweera, 1982; Ahmad *et al.,* 2019). Neem oil is the extract from the seed of the

neem tree. It has a strong odor and bitter taste due to volatile sulphur compounds and toxic fatty acids such as nimbin, nimbinin, nimbidin and nimbidiol (Biswas *et al.,* 2002). In many Asian countries, including Sri Lanka, external application of this oil has been used as a traditional remedy. Neem leaves and turmeric are used for many skin diseases and skin care remedies. Fresh leaves' antiseptic properties are commonly utilized for washing wounds, ulcers, and baths for patients recovering from chicken fox and childbirth. Even the juice of fresh neem leaves is given with rock salt to control intestinal worms as a remedy (Fernando, 1993). Further, *Azardirachta indica* is a useful insecticide for integrated pest management in organic farming systems.

24. Brindle berry/Goraka (*Garcinia zeylanica;* Family- Clusiaceae)

Garcinia zeylanica is an endemic glabrous tall tree with dark-colored bark and spreading branches. Fruit, leaves and bark are used. Seeds are embedded inside a soft juicy acidic fruit. In Sri Lanka, sun dried and smoked fruits of *Garcinia zeylanica* are extensively used as culinary spices. In indigenous medicinal practices, *Garcinia zeylanica* is used in many remedies for weight loss, cholesterol control, fractures and wounds (Jayaweera, 1980). Phenols, flavonoids, alkaloids, saponins, steroids are found in *Garcinia zeylanica* (Hewageegana *et al*., 2018; Patil and Appaiah, 2015) which may facilitate those medicinal properties. People take *Garcinia zeylanica* as a remedy for weight loss, exercise performance, joint pain, bloody diarrhea, to increase bowel movements, and for treating worms and parasites. Several health claims are made about *Garcinia zeylanica* extracts among Sri Lankan people such as diabetes, cancer, ulcers, diarrhea, and constipation (Jayaweera, 1980; Hewageegana *et al*., 2018). Extracts of the plant have been used in various folk medicines such as remedies against helminthic, protozoal and bacterial infections due to its unique composition and antimicrobial properties (Hewageegana *et al.,* 2018; Jayaweera, 1980; Nirasha *et al*., 2020).

25. Jungle geranium/ Ixora (*Ixora coccinea;* Family – Rubiaceae)

*Ixora coccinea*is a small shrub about 2-6 m tall which bears flowers in inflorescences with few branches. Flowers, fruits and bark are used for various purposes. Flowers are red, pinkish, or white. The fruit is edible while leaves, bark and flowers have medicinal properties. Traditionally *Ixora* is found to be useful for many common ailments such as hepatic disorder, pains, cancer and microbial infection. Various medicinal properties of this plant are documented and have been reported to possess different classes of chemical compounds including triterpenoids, aromatic acrid oils, tannins, saponins, carbohydrate, fatty acids, flavonoids and sterols (Dontha *et al*., 2015). *Ixora* is used for treating skin diseases, eye diseases, candida infections (Jayaweera,1982). Several *Ixora coccinea* species are used as astringent and to treat dysentery and tuberculosis. An infusion of the leaves or flowers of several species is administered to treat fever, headache and colic. Further, the decoction of the roots is used as a sedative. The external applications are based on *Ixora* plant's astringent and antiseptic properties. Its flowers are used to enrich medicinal baths/ Ayurveda baths along with red sandalwood *(Pterocarpus santalinus)*,veniwal (*Coscinium fenestratum*) or with neem leaves by Sri Lankans (Fernando, 1993; Jayaweera, 1982).

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Review Article

Pharmacological potential of wood apple (*Limonia acidissima*): A Review

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ABSTRACT

Wood apple is an edible fruit of Rutacae family with less exploitation. The fruit pulp is edible and used in many food preparations around the globe. It is also used long time as traditional medicines. This review aimed to summarize the pharmacological potentials of this fruit. This fruit is effective against some deadly disease such as cancer, diabetes, hyperlipidemia and microbial infection. It also has capability to treat diarrhea, ulcer, and pain. Both ripe and unripe fruit has strong pharmacological activity. The presence of phenolic and flavonoids are the main antioxidant responsible for the therapeutic potentials.

Keywords: Antimicrobial, cancer, diabetes, pharmacological activity, wood apple

INTRODUCTION

Plants are one of the major source traditional medicines which have been used more than thousands of years ago. Natural plant based products has already gaining popularity because of its low side effects and its popularity is rising with widening range of applications from pharmaceuticals to cosmetics. The Medicinal & Aromatic Plants are main sources of different phytochemicals – alkaloids, phenolics, saponins, steroid, flavinoids, glucoside, terpenoids, tannins, aliphatic alcohols, acids and esters, etc. and essential oils with a wide variety of applications in pharmaceuticals, flavours and fragrance, disinfectants, oral hygiene and in almost all spheres of human activity (Sarwar, 2020). The fruit wood apple (*Limonia acissimia* L) belongs to the family of Rutaceae and is found in India, Pakistan, Bangladesh, Sri Lanka and Southeast Asian countries (Rodrigures *et al.,* 2018; Hiwale, 2015). The fruit resembles an apple which leads to the establishments of the name. The peculiar fruit wood apple looks like a rotten coconut on the shell while in the inside it has soft pulp. The fruit smells like mixed stench of rotten blue cheese with overripe bananas. Wood apple pulp has sour and funky taste which is delicious with touch of sugar in desserts or with warm spices for savory dishes. The fruit is a remedy for cancer, diabetes, diarrhea, ulcer, and blood pressure. Regular consumption of this fruit helps to prevent from these disease.

PHYTOCHEMICALS AND NUTRIENTS

Wood apple contains phytochemicals like polyphenols, vitamins, saponins, coumarins, amino acids, tri-terpenoids, phytosterols and tannins (Pandey *et al*., 2014). Phytochemical analysis of *Limonia acidissima* ripe fruits indicates presence of flavonoids, steroids, glycosides and various acidic compounds. The major chemical compounds in leaf are acidissimin and acidissiminol. Presence of alkaloids, phenolsresins, gum and mucilage, fixed oils and fats are also noted in leafs (Aneesha *et al.,* 2018; Panda *et al*., 2013; Jayashree and Londonkar, 2014 and Vijayvargia *et al*., 2014).The wood apple pulp is very good source of carbohydrates (70.14%), protein (13.8%), fat (4,3%) and dietary fibre (1.7%) (Pandey *et al*., 2014; Asp, 1996). Presence of low amount of fat (4.38%), calcium, magnesium, iron, and high amounts of zinc are also reported in this fruit. High amount of phosphorous and calcium are also found out that exerts vital role in bone formation, blood clotting and more other metabolic processes (Table 1). The presence of iron in fruit indicates effectiveness against anemia, tuberculosis and other disorders (Campous *et al*., 2009).

ETHNOMEDICINAL USES

Various parts of wood apple have been used for more than thousands of years in traditional medicines. Both ripe and unripe fruits have reputation for its medicinal properties. The phytochemical and minerals are responsible for

Table 1: Mineral and Vitamins content of kaitha pulp (Pandey *et al*., 2014)

Minerals (ìg/g)			
Analyte	**Concentration(ìg/g)**	**Analyte**	**Concentration(ìg/g)**
P	1137.35	Cr	1.543
Mg	852.5	Pb	0.163
Ca	711.8	Li	0.241
Fe	23	Mo	0.263
Zn	23.84	Ni	0.819
Cu	6.67	Se	0.768
Mn	3.64	Ti	0.257
Vitamins(ìg/g)			
Vitamin C	180	Thiamine(B_1)	0.31
Riboflavin(B_2)	0.23	Beta-carotene	0.04

providing therapeutic roles. In traditional system it is used to cure dysentery, diarrhea, asthma, wounds, tumors, hepatitis and cardiac debility. Ripe fruit also cure liver disease and heart problems. It has role on lowering cholesterol levels in blood (Vidhya and Narain, 2011 and Mishra and Garg, 2011). Juice of wood apple (50mg/ L) with warm water and sugar is recommended for detoxification via blood purification and removal of toxins from the body (Vasant and Narasimhacharyaa, 2011). In children, juice of wood apple leaves mixed with milk and sugar is given for the remedy of biliousness and intestinal problems. The essential oil of crushed leaves is used to cure itching and improves digestion problem (Morton, 1987). Wood apple leaves contains high amounts of tannins which is effective against peptic ulcer. The leaves are also effective in the treatment of breast cancer, uterus cancer, infertility, progesterone deficiency, flu and respiratory disorders (Jayakumar and Geetha, 2012). The leaves have traditional use in snake bites and against bacterial pathogens (Kirtikar and Basu, 1995). Astringent, carminative and hepatoprotective activity are also known for leaves (Ilango and Chitra 2009).The pulp has low fat content (4.38%) and is an effective diet for overweight people (Pandey *et al*., 2014). The fruits are refrigerant, stomachic, stimulant, diuretic, astringent, aphrodisiac, cardiac tonic, liver tonic, anti-asthmatic, antidiarrheal, leucorrhoeal and also effective against dysentery. The seeds are used in the treatment of heart diseases (Jadeja *et al*., 2005; Senthilkumar *et al*., 2010). Spine of the tree is a remedy of menorrhagia. The bark contains 0.016% marmesin, aurapten, bergapten and other coumarins and is applied on venomous wounds upon crushing (Morton, 1987). The gum has demulcent, constipating, anti-diarrheal and anti-haemorrhoidal properties (Jayakumar and Geetha, 2012).

PHARMACOLOGICAL PROPERTIES

Anticancer activity

Essential oils isolated from leaves of wood apple shows antioxidant and cytotoxic activities. Thirugnanasampandan and David (2014) demonstrated that essential oil of 89.19µg/ml inhibits human cancer cell line MCF-7 (Michigan Cancer Foundation-7) by DNA fragmentation. In the same ways Pradhan *et al.* (2012) evaluates antineoplastic activity of ethanolic extracts of fruits on human breast cancer cell lines (SRBR3 and MDA-MBA435) and found effective dose ED50 of 56.1 and 30.6ìg/ml respectively. Eluru *et al.* (2015) reveals the in vitro anti-tumor activity of methanolic extracts of fruits at oral dose of 570mg/ kg body weight on mice model of Dalton's Ascitic Lymphoma (DAL) cell found that treatment with extract enhance nonviable cell counts in peritoneal exudates and decrease the viable cell count which may be because of absorption of extract by viable cells and the ultimate results was cell lysis by

activation of macrophage or any type of cytokine production. The extract also restored RBC and hemoglobin content (Eluru *et al*., 2015). The anticancer property of the fruits is may be due to the presence of flavonoids (Ilango, and Chitra, 2009).

Antidiabetic activity

Anti-diabetic activity of 95% ethanolic extracts of unripe wood apple fruits was evaluated on streptozotocin-induced diabetic rats at 250mg/kg body weight and found that it considerably lowers blood glucose levels of fasted, fed, and streptozotocin-induced diabetic rats (Gupta *et al*., 2009). Hypoglycemic effects are also observed on alloxan-induced diabetic rats. Methanolic extract at 1.75g/kg body weight is effective in prevention of hyperglycemia (Mishra and Garg, 2011). A 21 day trial of wood apple bark at dose of 200mg/kg and 400 mg/kg body weight of methanolic extract reduces blood glucose level by 39% and 54.5% respectively (MohanaPriya *et al.,* 2012). A similar effect was found by alloxan induced rat which was measured by blood serum levels (Ilango and Chitra, 2010). Anitha *et al*. (2015) also determined significant lowering of fasting and post prandial blood sugar level after consumption of fruit juice for 90 days.

Antihyperlipidemic activity

After administrating fruit powder at 2.5, 5 and 10 g/kg body weight for 28 days reduces lipid profile, hepatic glucose-6-phosphatase, and significant increases hepatic glycogen, hexokinase and HDL. The presence of fibres, phytosterols, saponins, polyphenols, flavonoids and ascorbic acid may be responsible for that (Rupal *et al*., 2013).

Antioxidant activities

Antioxidant activity of wood apple pulp was determined by water, petroleum ether, chloroform, ethyl acetate, and methanol extracts by Priya Darsini *et al.* (2013). They conducted 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, trolox equivalent antioxidant capacity (TEAC) assay, hydroxyl radical scavenging assay (HRSA), ferric reducing antioxidant power (FRAP) assay, nitric oxide radical (NO) scavenging assay, and total antioxidant activity (TAA) and found marked antioxidant activity. In-vitro antioxidant activity of Petroleum ether, chloroform and methanolic extract of wood apple leaf was determined by DPPH radical scavenging activity, Nitric oxide radical scavenging activity and hydrogen peroxide scavenging activity method and reveals that all the extract possesses sufficient antioxidant property (Attarde, 2016). Similar activity was also reported by Kerkar *et al.* (2020) and Singhania *et al.* (2020). Methanolic extract was also effective in synergizing antioxidant enzymes such as superoxidase dismutase and catalase (Patel *et al.* 2012). A 30 days oral administration of ethanolic extract of leaf at 200mg/kg body weight was effective in increasing activity of enzymatic antioxidants like Glutathione, superoxide dismutase (SOD), catalase (CAT) and peroxidase (Balamuruganvelu *et al.,* 2015).

Hepatoprotective activities

Hepatoprotective activity of methanolic extracts of pulps was evaluated by Ilango and Chitra (2009) against carbon tetrachloride (CCl4)-induced liver damage in rats. They found that levels of hepatic enzymes especially aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (AST), total protein (TP), total bilirubin (TB), and gamma glutamyltransferase (GGT) was increased and levels of super oxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione (GSH) decreased by treatment of 100, 200 and 400mg/kg i.p. for 3 days in rats. In another study aqueous leaf extract raises serum alanine transaminase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) level. It also elevates urea, creatinine, potassium and sodium levels in the phenyl hydrazine-induced anemic non-treated rats compared to the normal control (Anacletus *et al*., 2019).

Diuretic activity

The methanolic extract of leaves has significantly increase urine output. The extent of urination and electrolyte excretion namely Sodium, potassium and chloride ions depend on extraction method. It was found that Microwave assisted extraction (MAE) has better activity as compared to Bath Sonicator extraction (BSE)(Parial *et al*., 2009).

Antiulcer and wound healing activities

It was found that that wood apple fruit pulp is effective against indomethacin-induced gastric ulcer in rats. At 500mg/kg it inhibits gastric ulceration by reducing gastric HCl concentration through increasing intra-gastric pH (Mishra *et al*., 2009). Wound healing activity of methanolic fruit extract increased by tightening wound-breaking strength, decreased epithelization period, increased wound contraction, and increased granulation tissue weight and hydroxyproline concentration at 400 mg/kg of the extract (Ilango and Chitra, 2010). *L. acidissima* extracts significantly protect the gastric mucosa against ethanol induced injury by the reduction in the mucosal lesions on dose dependent manner. The phenolic compounds presents in the fruit is responsible for protection against ulcer on gastric wall and leucocytes infiltration of submucosal layers at 400 mg/kg leaf extract (Aneesha *et al*., 2018).

Analgesic activity

The analgesic activity was found against acetic acid- induced writhing mice and found 60.53% on methanol, 59.65% on acetone extracts of fruit peel as against 78.07% on standard drug Diclofenac Na (Islam *et al*., 2020).

Neuroprotective activities

Neuroprotective effects of wood apple was evaluated and showed that at 250 mg/kg and 500mg/kg body weight it inhibits ischemia reperfusion-induced brain injury in rats (Rakhunde *et al.,* 2014).

Spermatotoxic activities

Dhanapal *et al*. (2012) studied antispermatogenic activities of wood apple fruit pulp in adult male rats by treating with ethanolic extracts at 250 and 500mg/kg for 55 days and reveals that they were responsible for decline in sperm count, motility and viability. They also increased proportion of abnormal sperm and reduce testicular protein content by 24.58% and 29.86%, respectively.

Antidiarrheal activity

Senthilkumar *et al*. (2010) determined antidiarrhoeal and gastrointestinal motility reducing activity on aqueous bark extract of wood apple and found marked antidiarrheal activity by reducing average faeces weight and reduce GI motility (Senthilkumar *et al*., 2010). Similar data was found by Thomas method on castor oil-induced diarrhea at 500mg/kg of methanol and acetone peel extracts and observed 47.13% and 44.83% inhibition (Islam *et al*., 2020).

Antimicrobial activity

The methanol and acetone fruit peels extract at 250mg/kg shows moderate activity which inhibits 34.45% and 35.63% on *Klebsiella Oxytoca*, *Vibrio metschnikovii*, *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus* (Islam *et al*., 2020). The essential oil present on wood apple leaves containing β-pinene (28.4%), Z-anethole (22.1%), methyl chavicol (12.0%), and E-anethole (8.1%) which exerts antibacterial activity against five Gram-positive (*Staphylococcus aureus*, *Micrococcus flavus*, *M. luteus*, *Bacillus subtilis*, *Streptococcus faecalis*) and eight Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumonia*, *Serratiamarcescens*, *Proteus mirabilis*, *P. vulgaris*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Enterobacteraerogenes*), and four fungi (*Aspergillusniger*, *A. fumigatus*, *Penicilliumchrysogenum*, *Candida albicans*). The oils are also effective against *Micrococcus luteus*, *Proteus mirabilis*, *Penicillium chrysogenum*, and *Aspergillus niger* with minimum inhibitory concentration values of 0.31, 0.52, 0.20, and 0.26 mg/ml, respectively (Joshi *et al*. 2011). Antibacterial activity was also assessed by agar well diffusion method against three gram positive bacteria (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis*) and a gram negative bacterium (*Proteus mirabilis*) (Pandey *et al*., 2014). The essential oils are present in different parts of the plant and provides antibacterial activity (Bagul *et al*., 2019). Minimum inhibitory concentration (MIC) was also determined by Naidu *et al.* (2014) on leaf extract in hexane, chloroform and methanol and show sufficient activities. Biosynthesized silver and zinc oxide nanoparticles was tested against *Staphylococcus aureus*, *Bacillus cereus*, *Enterococcus faecalis*, *Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa* and found

effectiveness (Patil and Taranath, 2018). Antimicrobial activity was also determined by well diffusion method against *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia* (Sonawane *et al*., 2018). There is another study of silver and zinc oxide nanoparticles on *S. aureus*, *S. typhi* and *P. aeruginosa* at 400 µg/ml and found maximum zone of inhibition 15.16, 15.5 and 13.33/ mm respectively. Although zinc oxide Nano particles shows less activity as by comparison with silver Nano particles (Bheemanagouda *et al*., 2018). These Nano particles are also effective against *M. tuberculosis* at 12.5 ìg/ml (Bheemanagouda *et al*., 2016). The wood apple is used traditionally in Thailand to treat oral and throat infection (Lairungruang *et al*., 2014). Pandey *et al.* (2014) evaluates antibacterial activity of dried pulp and rind at 500 mg/ml on *Staphylococcus aureus*, *Staphylococcus, epidermidis*, *Bacillus subtilis* and *Proteus mirabilis*. Other researchers are also reported antibacterial activity of wood apple (Panda *et al*., 2013; Momin *et al*., 2013 and Naidu *et al*., 2014).

Anti-fungalactivity

Pradhan *et al.* (2012) claims that fruit shells of wood apple has antifungal compounds such as psoralene, xanthotoxin, 2, 6-dimethoxy benzoquinone, and osthenol . Antifungal activity of leaf ethanolic extracts was done by cup plate techniques on *M. gypseum*, *T. tonsurans*, *T. mentagrophytes*, *C. albicans* and least *T. rubrumon* dose dependent manner (Shivakumar and Vidyasagar, 2015).

Larvicidal activities

Wood apple leaf is effective against larvae of *Culexquinque fasciatus* with 90% mortality at 3% aqueous extract. The chloroform and methanol extract also shows 95% mortality at 100 ppm concentration (Banerjee *et al*., 2011). Mosquitocidal activity was found due to the presence of terpene in wood apple leaf which inhibits eggs, larvae, and pupae of *Aedesaegypti*. The extracts have very good larvicidal and pupicidal activity (Reegan *et al*., 2014). Hexane extract of *L. acidissima* shows ovicidal activity 79.2% and 60% at 500 ppm concentration against the eggs of *Cx. quinquefasciatus* and *Ae. Aegypti* (Reegan, *et al*., 2015).

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Evaluation of postharvest quality and shelf life of rose apple (*Syzygium jambos* L.) during storage

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ABSTRACT

Three rose apple accessions and five postharvest bagging treatments viz., Control (non-bag), unperforated low density polyethylene (LDPE) bag, perforated LDPE bag, unperforated brown paper bag, and perforated brown paper bag were evaluated for storage quality and shelf life of rose apple. Results showed that different postharvest treatments significantly influenced fruit quality and shelf life of rose apple. Changes in skin colour, weight loss, moisture, dry matter, vitamin C and pH of LDPE bagged fruits irrespective of perforation were slower than non-bagged control fruits during storage at ambient condition. The shelf life of fruit was counted until two weeks of storage. It was found that fruits stored in unperforated LDPE bag exhibited the longest shelf life (14.40 days) as compared to other treatments. The overall results expressed that rose apple quality can be maintained effectively at least six days using various bagging materials. However, it may conclude that unperforated LDPE bag could be used for extending storage life with maintaining external and internal qualities of rose apple.

***Keywords*:** Minor fruit, packaging, postharvest, rose apple, shelf life, storage

INTRODUCTION

Rose apple (*Syzyzium jambos* L.) locally known as *Golapjam*, is a non-climacteric tropical fruit belongs to the family Myrtaceae. It is an underutilized and neglected minor fruit in Bangladesh. It is an excellent fruit and appeals everybody for its nice rosy fragrance, good and spongy texture which is neither very soft nor very hard and very low acerbity. It is also known as plum rose, Malaya apple, Malabar plum, Yambo (Kishore *et al.*, 2016). It is native to Eastern India and a popular subtropical minor fruit which is not fully exploited. In Bangladesh, rose apple is found to grow all over the country due to its attractive fruits liked by the children or planted as an ornamental tree because of its sensational foliage and flowers. Rose apple fruit is rich sources of fat, vitamins and minerals. It contains plenty of sugars, total soluble solids, carotene and anthocyanin. Ripe fruit can be used to make rosewater. Rose apple fruit also contained different volatiles compounds (Guedes *et al*., 2004) which are used in the cosmetic industry. The fruits are used to make jam, jelly with lemon juice added or more frequently used as flavouring agent. It is also made into syrup for use as flavour in cold drinks. However, this fruit is mostly consumed as a table purpose fruit.

Since rose apple is a non-climacteric fruit (Das *et al*., 2011), therefore this fruit is harvested after ripen on the tree. It is very critical to retain freshness after harvest as its skin is very thin and soft textured flesh therefore moisture is quickly lost from fruits and rapidly deteriorates the quality (Plainsirichai *et al*., 2010; Das *et al.,* 2011). As a result, growers are bond to compulsorily market this fruits on the day of harvest, but long distance marketing is a great problem due to loss of freshness. Therefore, technologies towards postharvest loss reduction are necessary in form of extending shelf-life of rose apple fruit. Reducing moisture loss from fruit surface may assist in extending postharvest life of any fruit. Vapour pressure deficit between the fruit and its surrounding atmosphere should be reduced to prevent transpiration water loss from fruit surface thus preventing weight loss and delaying shrinking. In this case modified atmosphere packaging (MAP) can be used to handle this condition to extent the storage life of rose apple. A number of researches on MAP for extending shelf life and postharvest qualities of fruits and vegetables have been done (Hassan *et al*., 2005, Soltani *et al*., 2015). Although low temperature storage can extend the shelf life horticultural commodities but this process is costly.

Therefore, low cost modified atmosphere packaging storage in an ambient condition could be a useful technology for extension of shelf life of rose apple by maintaining physical and biochemical traits of fruits and it may help to long time marketing. The aim of this study was to evaluate the postharvest quality and shelf life of rose apple under different packaging materials in ambient storage condition

MATERIALS AND METHODS

Experimental materials

This study was conducted during February to May 2019. Ripe fruits of three rose apple accessions were harvested from Bangladesh Agricultural University Germplasm Centre (BAU-GPC). After harvest, fruits were immediately transferred to the Postgraduate laboratory of the Department of Horticulture, BAU.

Experimental design and treatments

The two-factor experiment was carried out following completely randomized design with three replications. The study comprised of three rose apple accessions namely ACS SJ_1, ACS SJ_2, ACS SJ_3 and five postharvest packaging treatments *viz.,* T_o: Control (Non-bag), T_1: Fruit stored in unperforated low density polyethylene (LDPE) bag, T_2: Perforated LDPE bag, T_3: Unperforated brown paper bag, and T_4: Perforated brown paper bag. The perforation size was 2 cm in diameter. After application of treatments, fruits were kept on a brown paper in the laboratory floor at ambient condition. Physio-chemical changes and shelf life of fruits were investigated at different days after storage (DAS).

Fruit skin colour changes during storage was determined using numerical rating scale of 1-5, where 1= Light yellowish green, 2= Light yellow, 3=Yellow, 4= Light yellow brown, 5= Yellow brown.

Percent weight loss of fruits of each treatment was measured by using electric weighing balance. Percent total weight loss was calculated by using the following formula:

$$\% \text{ weight loss} = \frac{\text{Initial weight (g) -Final weight (g)}}{\text{Initial weight (g)}} \times 100$$

Determination of moisture and dry matter contents

Ten grams of fruit pulp was weighed in a porcelain crucible (which was previously cleaned, dried and weighed) from each treatment and replications. The crucible was kept in electric oven at 65°C for 72 hours until the weight became constant. It was then cooled in desiccators and weighed again. Percent moisture and dry matter contents were calculated by using the formula:

$$\% \text{ moisture content} = \frac{\text{Fresh weight of sample (g)} - \text{Dry weight of sample (g)}}{\text{Fresh weight of sample (g)}} \times 100$$

$$\% \text{ dry matter content} = \frac{\text{Dry weight of sample (g)}}{\text{Fresh weight of sample (g)}} \times 100$$

Total Soluble Solids (TSS)

TSS content of rose apple pulp was estimated by using Abbe's refractometer (ATAGO Company Ltd., Japan). A drop of rose apple juice squeezed from the fruit pulp on the prism of the refractometer. Percent TSS was obtained from direct reading of the instrument. Temperature corrections were made by using the methods described by Ranganna (1995).

Vitamin C (Ascorbic acid)

Ascorbic acid content was determined according to the method of Plummer (1971). In brief, ten grams of fresh fruits and 70 ml 6% meta phosphoric acid solution was taken in a blender and homogenized for 2 minutes. Thereafter, it was filtered and centrifuged at about 2000 rpm for 5 minutes. The supernatant was transferred to a 100 ml volumetric flask and made up to the volume

with 6% meta phosphoric acid. Five ml of the aliquot was taken in a conical flask and titrated with dye solution. Ascorbic acid content was calculated by using the following formula.

$$\text{Ascorbic acid content } (\text{mg}/100\text{g}) = \frac{\text{Titer} \times \text{Dye factor} \times \text{Volumn made up (ml)}}{\text{Volumn of extract (ml)} \times \text{Weight of sample (g)}} \times 100$$

Fruit pH

Fruit pH was determined by using a glass electrode pH meter (Senso Direct pH 110, UK).

Shelf life

Shelf life is the period of time which started from harvesting and extends up to the start of rotting of fruits. Shelf life of rose apple fruits was estimated by counting the days required to storages to retaining, optimum marketing and eating qualities.

Statistical analysis

Data obtained from this study were analyzed to find out the significance of difference among the treatments. Data on different parameters were statistically analyzed using MSTAT C Statistical Package Program. The means for all the treatments were calculated and analysis of variances (ANOVA) for all the parameters was performed by *F*-test. The significance of difference between the pair of means was compared by least significant difference (LSD) test at the 1% and 5 % levels of probability.

RESULTS AND DISCUSSION

Changes of skin colour

Fruit skin colour is one of the important criteria to determine the quality of fruits as well as consumer acceptance. Skin colour of fruits significantly changed up to 4 days after storage (DAS) due to the influence of different rose apple accessions. It was found that fruit skin colour changed from light yellow green to yellow colour after six days of storage (Table 1). This trait is also influenced by different postharvest packaging treatments. It was noticed that the changes of skin colour were quicker in fruits under non-bag control treatment while colour changes were slower in fruits stored at unperforated brown paper bag (Table 2). In combination of rose apple accessions and packaging treatments it was observed that rapid colour change exhibited when ACS SJ_2 stored non-bag control treatment and the minimum colour change found in treatment combination of ACS SJ_1 stored in unperforated brown paper bag (Table 3). Charoenchongsuk *et al.* (2015) reported that skin colour changes during ripening of fruits due to alteration of chlorophyll. Chlorophyll degradation is a key factor of changes in pigment compassion that typically occur in fruit peel at the onset of ripening.

Weight loss

The variation in percentage of total weight loss was highly significant due to the effects of different accessions of rose apple at different days of storage. It was observed that fruit weight loss was faster in ACS SJ_2 while it was slower in ACS JS_1 during the entire storage period (Fig. 1a). Postharvest packaging treatments exerted significant effects on weight loss of fruits. Results showed that percent weight loss of fruits was steady during storage period under perforated and unperforated LDPE bag treatments. Fruit weight loss was accelerated in non-bag control treatment followed by perforated brown paper bag (Fig. 2b). The combined effects showed significant influence on total weight loss of fruits during storage. The rate of weight of loss was the fastest in ACS SJ_2 when stored in non-bag control treatment. In contrast, the changes were the slowest in ACS SJ_1 held in unperforated LDPE bag (Table 4). Plainsirichai *et al*. (2014) reported that coating the fruits of rose apple cv. Tabtim Chan with 2% chitosan and stored at an ambient temperature of 30°C resulted in significantly less weight loss, disease incidence and significantly higher fruit firmness compared to the control at day 5 of storage. In this study, we noticed that weight loss of rose apple fruits was the slowest from unperforated LDPE bagged fruits. Hagenmaier (2000) and Rojas *et al.* (2002) reported that texture and strength of citrus improved when fruit was coated, while uncoated fruits turn soft with the passage of storage duration. While Tefera *et al.* (2007) stated that the minimum weight losses occurred in unperforated LDPE bag wrapping fruits. They reported that polythene bag significantly reduced weight loss due to the inhibition of transpiration.

Table 1: Effects of different accessions on changes of skin colour at different days after storage

Accessions	Fruit skin colour change at different DAS			
	0	2	4	6
ACS SJ_1	1.00	1.62	2.22	3.02
ACS SJ_2	1.00	1.70	2.24	3.04
ACS SJ_3	1.00	1.70	2.18	3.03
$LSD_{0.05}$	-	0.03	0.04	0.05
$LSD_{0.01}$	-	0.04	0.05	0.07
Level of significance	ND	**	*	NS

** & * indicates significant at 1% & 5% levels of probability, NS = Not significant, ND= Statistical analysis not done

Table 2: Effects of postharvest packaging treatments on changes of skin colour at different days after storage

Postharvest treatments	Fruit skin colour change at different DAS			
	0	2	4	6
T_0:Control (Non-bag)	1.00	2.13	3.40	5.00
T_1:Unperforated LDPE bag	1.00	2.08	2.59	3.28
T_2:Perforated LDPE bag	1.00	2.15	2.36	2.57
T_3:Unperforated brown paper bag	1.00	1.00	1.50	2.13
T_4:Perforated brown paper bag	1.00	1.00	1.21	2.15
$LSD_{0.05}$	-	0.04	0.05	0.06
$LSD_{0.01}$	-	0.05	0.07	0.09
Level of significance	ND	**	**	**

** indicates significant at 1% level of probability, ND= Statistical analysis not done

Table 3: Combined effects of postharvest packaging treatments and accessions on changes of skin colour at different days after storage

Treatment combinations		Fruit skin colour change at different DAS			
		0	2	4	6
T_0: Control(Non-bag)	ACS SJ_1	1.00	2.00	3.25	5.00
	ACS SJ_2	1.00	2.21	3.58	5.00
	ACS SJ_3	1.00	2.17	3.38	5.00
T_1: Unperforated LDPE bag	ACS SJ_1	1.00	2.00	2.66	3.21
	ACS SJ_2	1.00	2.08	2.56	3.25
	ACS SJ_3	1.00	2.17	2.54	3.38
T_2: Perforated LDPE bag	ACS SJ_1	1.00	2.12	2.50	2.66
	ACS SJ_2	1.00	2.19	2.21	2.56
	ACS SJ_3	1.00	2.15	2.37	2.50
T_3: Unperforated brown paper bag	ACS SJ_1	1.00	1.00	1.17	2.00
	ACS SJ_2	1.00	1.00	1.50	2.21
	ACS SJ_3	1.00	1.00	1.40	2.17
T_4:Perforated brown paper bag	ACS SJ_1	1.00	1.00	1.60	2.21
	ACS SJ_2	1.00	1.00	1.25	2.17
	ACS SJ_3	1.00	1.00	1.20	2.08
$LSD_{0.05}$		-	0.07	0.09	0.11
$LSD_{0.01}$		-	0.10	0.12	0.15
Level of significance		ND	**	**	**

** indicates significant at 1% level of probability, ND= Statistical analysis not done

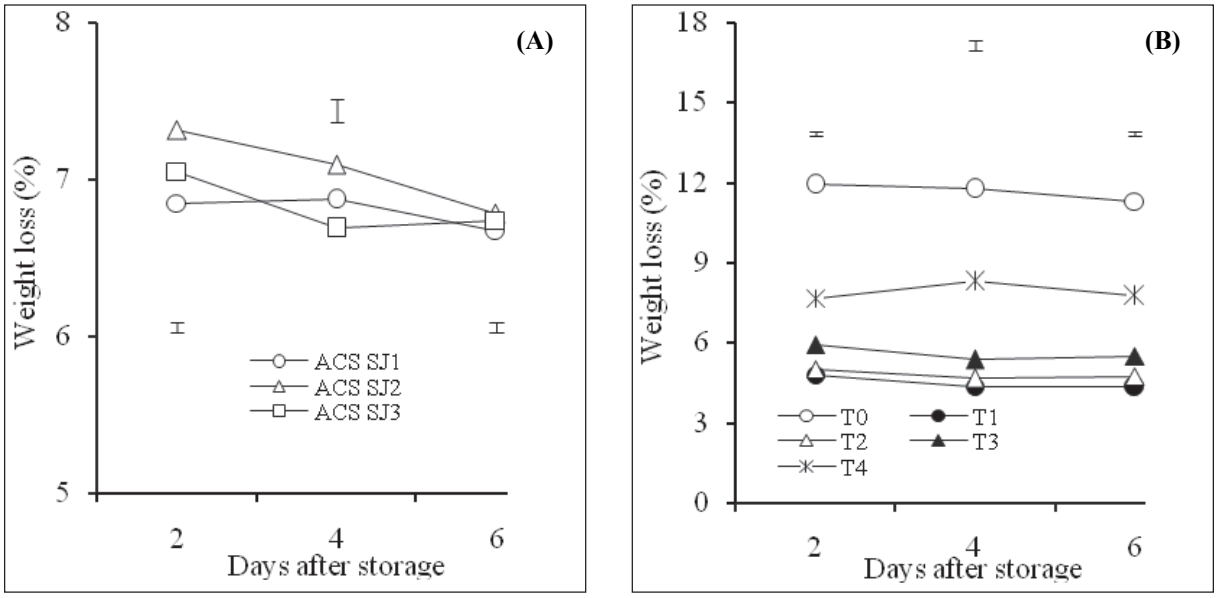

Fig. 1: Effects of rose apple accessions (A) and postharvest treatments (B) on weight loss at different days after storage. Vertical bars represent LSD at 1% level of significance. T_0: Control (Non-bag), T_1: Unperforated LDPE bag, T_2: Perforated LDPE bag, T_3: Unperforated brown paper bag, T_4: Perforated brown paper bag.

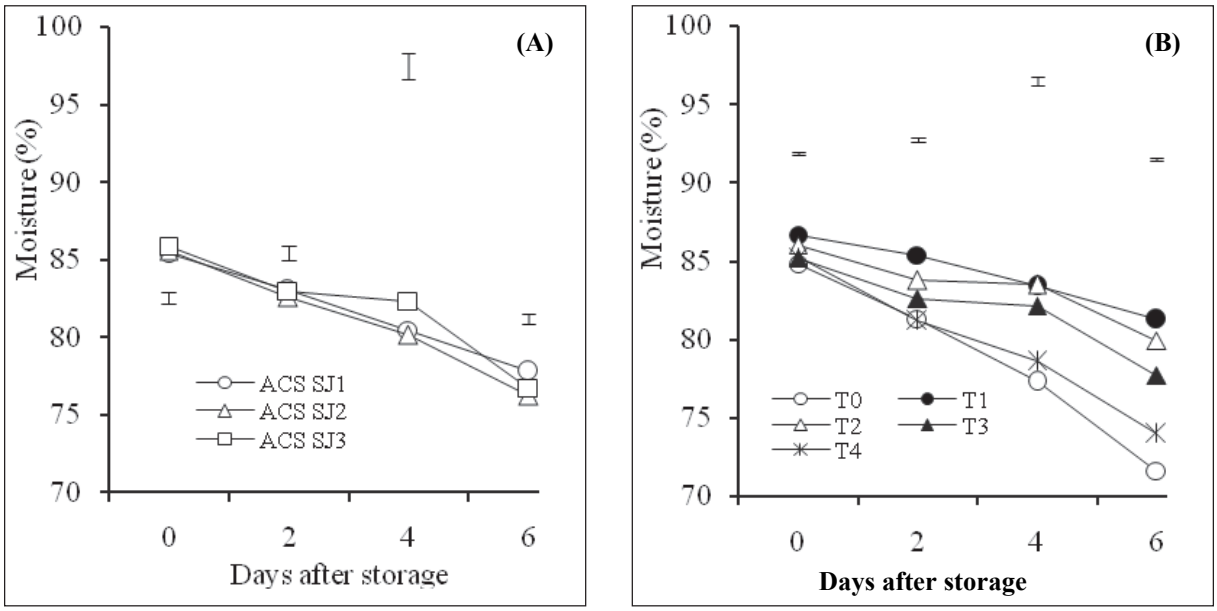

Fig. 2: Main effects of rose apple accessions (A) and postharvest treatments (B) on moisture content at different days after storage. Vertical bars represent LSD at 1% level of significance. T_0: Control (Non-bag), T_1: Unperforated LDPE bag, T_2: Perforated LDPE bag, T_3: Unperforated brown paper bag, T_4: Perforated brown paper bag.

Moisture content

Reduction of moisture content of rose apple during storage varied significantly due to the influence of different accessions. It was found that, all of the accessions lost moisture content during storage and this trend was faster in case of ACS SJ_2 fruits which lost 9.31% moisture while ACS SJ_1 lost 7.61% (Fig. 2a). Postharvest packaging treatments exerted significant effects on depletion of percentage of moisture content in fruits during storage. It is noticed that moisture content in fruits sharply declined when it was stored in non-bag

Table 4: Combined effects of postharvest packaging treatments and accessions on percent of weight loss and moisture content at different days after storage

Treatment combinations		Initial weight (gm)	Weight loss (%) at different DAS			Moisture (%) at different DAS			
			2	4	6	0	2	4	6
T_0: Control (Non-bag)	ACS SJ_1	16.90	11.30	12.01	11.43	82.99	80.70	76.38	72.06
	ACS SJ_2	18.25	11.49	12.98	12.51	86.00	81.25	77.52	71.25
	ACS SJ_3	17.98	10.98	10.32	11.98	85.48	82.05	77.98	71.56
T_1: Unperforated LDPE bag	ACS SJ_1	19.08	4.08	4.12	4.43	87.49	85.98	83.58	81.86
	ACS SJ_2	18.50	4.38	4.57	4.97	87.01	86.02	83.87	80.92
	ACS SJ_3	20.03	4.56	4.38	4.98	85.46	84.15	82.79	81.06
T_2: Perforated LDPE bag	ACS SJ_1	21.02	4.51	4.32	5.13	85.46	83.25	81.83	80.48
	ACS SJ_2	18.02	4.65	4.71	4.93	86.68	83.86	79.62	79.36
	ACS SJ_3	19.00	5.04	4.92	5.02	85.88	84.17	89.00	80.02
T_3: Unperforated brown paper bag	ACS SJ_1	17.89	6.01	5.43	6.14	86.14	84.25	82.68	79.83
	ACS SJ_2	18.50	5.38	5.16	5.43	84.34	81.23	81.69	76.32
	ACS SJ_3	21.00	5.12	5.46	6.23	85.13	82.38	82.05	77.08
T_4:Perforated brown paper bag	ACS SJ_1	19.50	6.73	7.06	6.95	84.86	80.92	77.83	74.68
	ACS SJ_2	18.90	8.35	9.47	9.25	83.65	80.48	78.02	73.28
	ACS SJ_3	19.75	7.02	8.35	7.98	87.43	82.17	79.88	74.05
$LSD_{0.05}$		-	0.19	0.45	0.19	0.56	0.69	1.23	0.50
$LSD_{0.01}$		-	0.26	0.61	0.26	0.76	0.93	1.66	0.67
Level of significance		ND	**	**	**	**	**	**	**

** indicates significant at 1% level of probability, ND= Statistical analysis not done

control (13.21%) and perforated brown paper bag (11.31%), respectively. The retention of moisture content was higher when fruits were stored in LDPE bag and unperforated brown paper bag (Fig. 2b). The loss of moisture content from fruits was higher when ACS SJ_2 stored in non-bag condition (14.75%) and the retention of moisture was higher when ACS SJ_3 fruits stored in unperforated LDPE bag which lost only 4.4% moisture during storage (Table 4). Naik *et al.* (1993) reported that polythene bag plays an important role in quality maintenance by slowing down the biochemical changes and reducing the moisture loss in fresh produces.

Dry matter content

Percentage of dry matter content of rose apple varied significantly among the accessions. It was observed that dry matter content increased as storage duration progressed. At 6th day of storage, fruits of ACS SJ_2 had the highest dry matter content (23.77%), while it was the lowest (22.22%) in ACS SJ_1 (Fig. 3a).

Different postharvest packaging treatments exerted significant influences on percentage of dry matter content in fruits during storage. At 6th day of storage, results revealed that dry matter content was the highest (28.38 %) in rose apple held in non-bag control treatment. On the other hand, it was the lowest (18.72%) in unperforated LDPE bag treatment (Fig. 3b). The combined effects of packaging treatments and accessions was also significant on dry matter content of rose apple. The maximum dry matter content (28.75%) was estimated in ACS SJ_2 under non-bag control treatment and the minimum (18.14%) recorded in ACS SJ_1 under unperforated LDPE bag treatment (Table 5).

Vitamin C (Ascorbic acid)

Vitamin C content of different accessions of rose apple declined with storage duration. However, at 6th day of storage, ACS SJ_3 contained highest vitamin C (17.30mg/100g) while ACS SJ_2 contained the lowest (14.86mg/100g) (Fig. 4a). Postharvest packaging treatments showed significant influences on vitamin C contents of rose apple during storage. Vitamin C content was reduced as storage period progressed in case of all packaging treatments. The reduction trend of vitamin C was faster in non-bagged control fruits

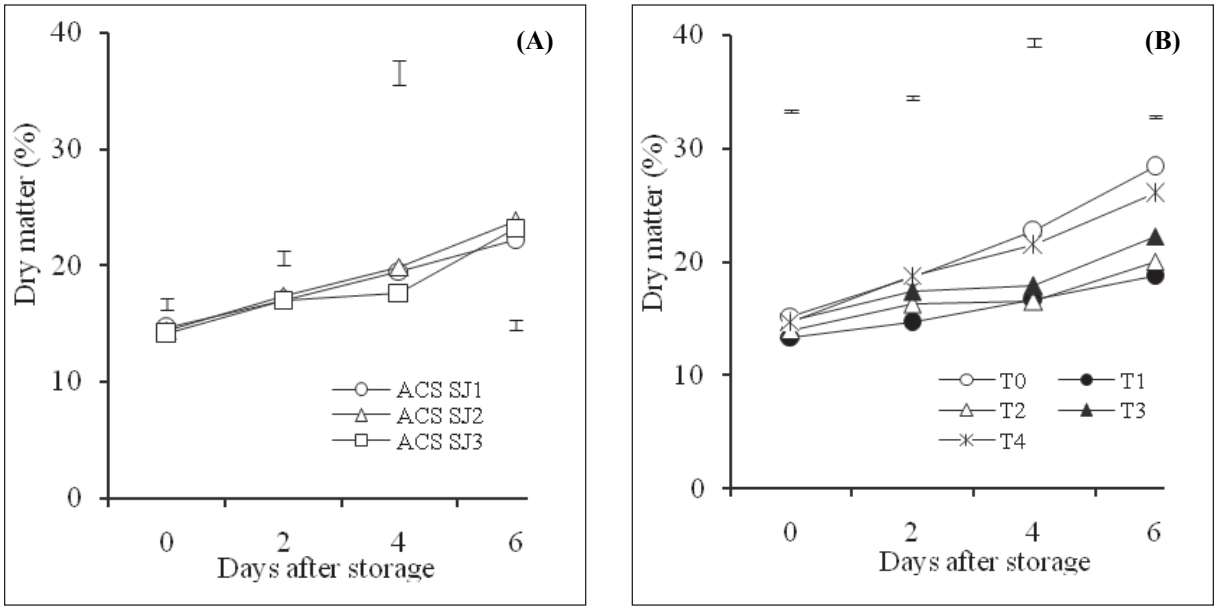

Fig. 3: Main effect of rose apple accessions (A) and postharvest treatments (B) on dry matter contents at different days after storage. Vertical bars represent LSD at 1% level of significance. T_0: Control (Non-bag), T_1: Unperforated LDPE bag, T_2: Perforated LDPE bag, T_3: Unperforated brown paper bag, T_4: Perforated brown paper bag.

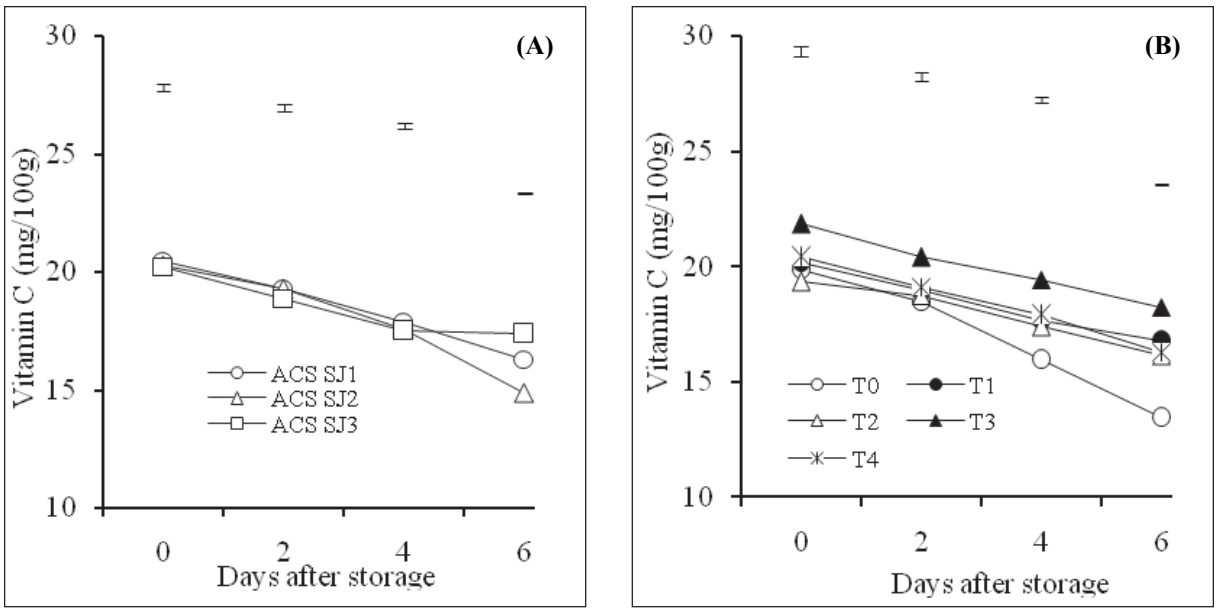

Fig. 4: Main effects of rose apple accessions (A) and postharvest treatments (B) on vitamin C contents at different days after storage. Vertical bars represent LSD at 1% level of significance. T_0: Control (Non-bag), T_1: Unperforated LDPE bag, T_2: Perforated LDPE bag, T_3: Unperforated brown paper bag, T_4: Perforated brown paper bag.

while it was slower in unperforated brown paper bagged fruits (Fig. 4b). The combined effects were also significant in relation to vitamin C contents. At 6th day of storage, the highest vitamin C (19.73 mg/100 g) was found in ACS SJ_3 treated with unperforated brown paper bag. In contrast, the amount of vitamin C (12.18 mg/100 g) was the lowest in ACS SJ_2 held in non-bag control condition (Table 5). Das *et al*. (2011) reported that ascorbic acid contents in rose apple increase up to two months after fruit set thereafter decreased as fruit become mature and start ripening.

Table 5: Combined effects of postharvest packaging treatments and accessions on dry matter and vitamin C contents at different days after storage

Treatment combinations		Dry matter (%)				Vitamin C (mg/100g)			
		at different DAS							
		0	2	4	6	0	2	4	6
T_0: Control(Non-bag)	ACS SJ_1	17.01	19.30	23.62	27.94	21.80	20.75	18.02	13.57
	ACS SJ_2	14.00	18.75	22.48	28.75	20.00	18.39	15.19	12.18
	ACS SJ_3	14.52	17.95	22.02	28.44	17.70	16.32	14.65	14.59
T_1: Unperforated LDPE bag	ACS SJ_1	12.51	14.02	16.42	18.14	19.50	18.02	16.28	17.25
	ACS SJ_2	12.99	13.98	16.13	19.08	20.00	19.13	18.29	15.05
	ACS SJ_3	14.54	15.85	17.21	18.94	21.00	19.85	18.35	17.95
T_2: Perforated LDPE bag	ACS SJ_1	14.54	16.75	18.17	19.52	18.00	17.57	16.00	16.45
	ACS SJ_2	13.32	16.14	20.38	20.64	20.50	20.02	18.85	15.02
	ACS SJ_3	14.12	15.83	11.00	19.98	19.50	18.59	17.32	16.95
T_3: Unperforated brown paper bag	ACS SJ_1	13.86	15.75	17.32	20.17	21.00	20.05	19.89	18.05
	ACS SJ_2	15.66	18.77	18.31	23.68	20.50	19.25	18.25	16.95
	ACS SJ_3	14.87	17.62	17.95	22.92	24.01	21.93	20.15	19.73
T_4:Perforated brown paper bag	ACS SJ_1	15.14	19.08	22.17	25.32	22.10	20.13	19.25	16.12
	ACS SJ_2	16.35	19.52	21.98	26.72	20.22	19.63	17.39	15.08
	ACS SJ_3	12.57	17.83	20.12	25.95	18.90	17.59	17.02	17.65
$LSD_{0.05}$		0.56	0.69	1.23	0.50	0.48	0.39	0.33	0.75
$LSD_{0.01}$		0.76	0.93	1.66	0.67	0.64	0.54	0.44	1.00
Level of significance		**	**	**	**	**	**	**	**

** indicates significant at 1% level of probability

Total soluble solids (TSS)

The difference in respect of TSS was found to be statistically significant during storage period of different accessions of rose apple. At 6th day of storage, ACS SJ_2 showed the highest TSS (11.87%) and ACS SJ_3 retain the lowest TSS (11.60 %) (Table 6). Postharvest packaging treatments also exerted significant effects on TSS content of rose apple during storage. Results showed that the TSS content was the maximum in unperforated LDPE bag. On the other hand, the value was the minimum in unperforated brown paper bag (Table 7). The combined effects were significant in relation to TSS content of fruit. The amount of TSS of rose apple fruit was higher (12.41%) in ACS SJ_2 when stored in unperforated LDPE bag. In contrast, the amount of TSS was lower (10.64%) in both ACS SJ_2 and ACS SJ_3 under unperforated brown paper bag (Table 8). TSS of fruits is increased as acidity decrease throughout the maturity of rose apple fruit (Das *et al*., 2011). Although internal quality of non-climacteric fruit did not change much but in our study we observed that at 6th day of storage, rose apple of ACS SJ_2 stored in unperforated LDPE bag showed higher level of TSS (12.41%) and ACS SJ_2 and ACS SJ_3 under unperforated brown paper bag showed lower TSS (10.64%). Caner *et al.* (2008) noticed that fruit TSS did not changed much during storage under modified atmosphere packaging.

Fruit pH

Fruit pH was slightly decreased during storage. Different accessions of rose apple exhibited significant effects on pH content of fruit during storage. Among the accessions, the reduction of pH was higher in ACS SJ_1 and lower in ACS SJ_2 (Table 6). Postharvest packaging treatments exerted significant effects on reduction pH of rose apple during storage. The reduction trend was higher in non-bag control treatment as compared to bagged fruits. The minimum pH was reduced in perforated LDPE and unperforated brown paper bagged fruits (Table 7). The combined effects of rose apple accessions and postharvest treatments were significant in relation to pH content of fruit. At 6th day of storage, pH was the higher in ACS SJ_1 held in unperforated brown paper bag (5.62). In contrast, the amount of pH was lower in ACS SJ_3 treated with the control treatment (5.21) (Table 8). Caner *et al*. (2008) reported that fruit pH did not change

during storage under different bagging treatments rather reduced reading recorded at the end of day 6 of storage. They also stated that fruit pH did not changed much during storage under modified atmosphere packaging.

Shelf life

Rose apple accessions performed slightly different in respect of shelf life. The longest shelf life was observed in ACS SJ_3 (10.46 days) followed by ACS SJ_2 (10.18 days) and the shortest shelf life was observed in ACS SJ_1 (10.00 days) (Table 6). Postharvest packaging treatments significantly influenced shelf life of rose apple. The longest shelf life (14.20 day) was found in fruits stored in unperforated LDPE bag followed by perforated LDPE bag (12.17 days), un perforated brown paper bag (10.23 days), perforated brown paper bag (8.23 days) and the lowest (6.23 day) in non-bag control treatments (Table 7). The combined effects of accessions and postharvest packaging treatments showed highly significant variations in respect of shelf life of fruits. The highest shelf life (14.40 days) was recorded in ACS SJ_3 stored in unperforated LDPE bag followed by the different accessions stored in perforated LDPE bag, unperforated brown paper bag, perforated brown paper bag, respectively and the lowest shelf life (6.00 days) was observed in ACS SJ_1 stored in non-bag control treatment (Table 8). LDPE bag restrict water loss from fruit which maintained internal and external qualities of fruit compared to control. Plainsirichai *et al.* (2010) reported that fruits treated with 1-MCP extended shelf life of rose apple. Fruit coating with 2% chitosan also increased shelf life of rose apple (Plainsirichai *et al*., 2014).

Table 6: Main effect of rose apple accessions on TSS, pH and shelf life at different days after storage

Accessions	TSS (%)				pH				Shelf life
	at different DAS				at different DAS				(days)
	0	2	4	6	0	2	4	6	
ACS SJ_1	11.37	11.27	11.60	11.71	5.58	5.51	5.47	5.43	10.00
ACS SJ_2	11.24	11.51	11.59	11.87	5.45	5.42	5.41	5.40	10.18
ACS SJ_3	11.07	11.35	11.51	11.60	5.45	5.41	5.40	5.38	10.46
$LSD_{0.05}$	0.17	0.14	0.05	0.07	0.04	0.05	0.05	0.04	0.03
$LSD_{0.01}$	0.23	0.19	0.07	0.10	0.05	0.07	0.07	0.06	0.05
Level of significance	**	**	**	**	**	**	**	**	**

** indicates significant at 1% level of probability

Table 7: Main effect of postharvest treatments on TSS, pH and shelf life at different days after storage

Postharvest treatments	TSS (%)				pH				Shelf life
	at different DAS				at different DAS				(days)
	0	2	4	6	0	2	4	6	
T_0:Control (Non-bag)	11.42	11.39	11.92	11.78	5.60	5.41	5.47	5.34	6.23
T_1:Unperforated LDPE bag	11.68	11.74	11.92	11.88	5.51	5.45	5.41	5.38	14.20
T_2:Perforated LDPE bag	10.11	10.60	10.81	11.65	5.46	5.43	5.40	5.38	12.17
T_3:Unperforated brown paper bag	11.19	11.36	11.40	11.50	5.50	5.48	5.45	5.42	10.23
T_4:Perforated brown paper bag	11.73	11.78	11.78	11.81	5.38	5.41	5.37	5.43	8.23
$LSD_{0.05}$	0.22	0.18	0.07	0.10	0.05	0.06	0.06	0.06	0.04
$LSD_{0.01}$	0.30	0.24	0.10	0.13	0.07	0.09	0.09	0.08	0.06
Level of significance	**	**	**	**	**	**	*	*	**

** and * indicates significant at 1% and 5% levels of probability

Table 8: Combined effects of postharvest treatments and accessions on TSS, pH and shelf life at different days after storage

Treatment combinations		TSS (%) at different DAS				pH at different DAS				Shelf life (days)
		0	2	4	6	0	2	4	6	
T_0: Control (Non-bag)	ACS SJ_1	11.25	10.12	11.50	11.45	5.83	5.75	5.62	5.56	6.0
	ACS SJ_2	12.00	12.16	12.24	12.09	5.65	5.58	5.54	5.49	6.2
	ACS SJ_3	11.00	11.90	12.01	11.80	5.32	5.28	5.25	5.21	6.5
T_1: Unperforated LDPE bag	ACS SJ_1	11.40	11.47	11.68	11.52	5.49	5.35	5.31	5.29	14.0
	ACS SJ_2	12.40	12.42	12.38	12.41	5.34	5.31	5.29	5.27	14.2
	ACS SJ_3	11.25	11.32	11.69	11.72	5.71	5.69	5.63	5.59	14.4
T_2: Perforated LDPE bag	ACS SJ_1	10.80	10.98	11.06	11.56	5.35	5.31	5.29	5.26	12.0
	ACS SJ_2	8.96	9.98	10.32	11.82	5.39	5.35	5.31	5.30	12.0
	ACS SJ_3	10.56	10.83	11.06	11.58	5.65	5.62	5.61	5.59	12.5
T_3: Unperforated brown paper bag	ACS SJ_1	11.00	11.34	11.38	11.63	5.67	5.65	5.64	5.62	10.0
	ACS SJ_2	12.11	10.58	12.21	10.64	5.43	5.41	5.38	5.35	10.2
	ACS SJ_3	10.56	12.17	10.62	10.64	5.39	5.38	5.33	5.31	10.5
T_4:Perforated brown paper bag	ACS SJ_1	12.40	12.43	12.40	12.37	5.54	5.51	5.48	5.42	8.0
	ACS SJ_2	12.38	12.39	12.37	12.39	5.42	5.38	5.32	5.30	8.3
	ACS SJ_3	10.41	10.51	10.58	10.66	5.18	5.35	5.31	5.29	8.4
$LSD_{0.05}$		0.39	0.32	0.12	0.17	0.09	0.11	0.11	0.10	0.07
$LSD_{0.01}$		0.52	0.43	0.17	0.23	0.12	0.15	0.15	0.14	0.10
Level of significance		**	**	**	**	**	**	**	**	**

** indicates significant at 1% level of probability

Conclusion

This study suggests that fruit quality and shelf life can be improved significantly by bagging fruits in unperforated LDPE bag compared to non-bag control fruits. From the above results it was found that the postharvest treatments caused significant effects on colour, weight loss, moisture, dry matter, vitamin C, TSS, pH and shelf life of rose apple. LDPE bag treated fruits gave the superior result in relation to the reduction of weight loss, vitamin C compared to other treatments, and which ultimately resulted in prolonged shelf life of rose apple. Therefore, it can be summarized that LDPE bag could be useful for storage of rose apple for at least two weeks after harvesting.

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Comparative accounts on proximate and phytochemical compositions and antioxidant properties of *Garcinia quaesita* and *Garcinia zeylanica.*

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ABSTRACT

Garcinia quaesita and Garcinia zeylanica are endemic to Sri Lanka and have been used in wide array of applications with respect to healthcare, food additives, food preservation, etc. nevertheless, the studies on phytochemical, proximate compositions, and antioxidant activity of their leaves have not been documented enough so far. Therefore, this study was aimed on those studies of both plants for the purpose of producing a scientific repository and to be used as support in transforming leaves into functional foods. Plant extraction was done using two techniques i.e., maceration and ultrasound-assisted-extraction and phytochemical screening and proximate composition were done following standard methods. Standard spectrophotometric methods were used for quantification of phytochemicals. DPPH and FRAP assays were used to measure the antioxidant capacity. The results of ultrasound-assisted-extraction showed as an efficient method for extraction of potential phytochemicals. Phytochemical screening reveals that most of the important phytochemicals are available in both garcinia varieties. The higher antioxidant capacity and acceptable proximate composition contain with both varieties showing that each has unique characteristic. The statistical analysis studies showed that G. quaesita is the best than G. zeylanica, showing the highest total antioxidant capacity of 225.63 ± 2.01mg Trolox Eq/g in FRAP assay and highest polyphenolic content (202.14 ± 2.27mg GAE/ g extract) and appreciable proximate composition. As a conclusion, this study provided the detailed analysis of phytochemical, proximate and antioxidant properties of the leaves of both garcinia varieties grown in Sri Lanka to be used by scientific community for further chemical analysis and applications in pharmacological aspects and value addition.

Keywords: Antioxidant, garcinia varieties, maceration, phytochemicals, proximate**,** sonication,

INTRODUCTION

Sri Lanka is one of the focused hot spots for its biodiversity in tropical rainforests where endemic terrestrial evergreen plant is recorded to be approximately 70 % (Gunatilleke *et al*., 2017). However, the potential use of Sri Lankan endemic plants in scientific world has not been in practice enough. In our previous study, we have reported on scientific studies of an endemic plant, *Dialiumovoideum thwaites* for its phytochemical compositions and antioxidant properties (Bulugahapitiya *et al*., 2020). *Garceinia quaesita* (Clusiaceae family) is a plant, endemic to Sri Lanka and locally referred as "Rath Goraka (Red-Goraka, Red fruited)" and commonly called as "Goraka" and Brindle berry in English. *Garcenia zeylanica* is also an endemic plant to Sri Lanka, which also belongs to the same family of *G. quaesita* and locally known as Ëla Goraka/Kaha Goraka (Yellow-Goraka, yellow fruited). The fruits of *G. quaesita* and *G. zeylanica* are used as a condiments or spice and importantly in folk/indigenous medicine. As a major-primary compound is known as hydroxycitric acid (HCA) in its fruits and rinds. Normally fruits are prescribed for ailments in Indian folk tradition and rinds are used as Sri Lankan curry ingredients and condiments (Farzana *et al*., 2010).

As literature on phytochemical, proximate compositions, and antioxidant activity of leaves are not available respect to above two endemic species, this study was aimed on comparative account on phytochemicals and proximate composition, quantification of total polyphenolic, flavonoid, tannin and terpenoid contents and antioxidant capacity of two garcinia varieties; *G. quaesita* and *G. zeylanica* for the purpose of producing a repository to be used for the scientific community and public and intension to replace the demand of Garcinia fruits with Garcinia leaves in pharmaceutical industry.

MATERIAL AND METHODOLOGY

Materials: Ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$ 98%), Aluminum chloride anhydrous ($AlCl_3$,98%), Dimethyl sulfoxide (DMSO,99.5%), Folin-Ciocalteu reagent, Sodium carbonate monohydrate ($Na_2CO_3 \cdot H_2O$,99.5%), Gallic acid monohydrate (98%), Tannic acid, Phosphomolybdic acid, 2, 22 -Diphenyl-1-Picrylhydrazyl Radical (DPPH*), 2,4,6-Tripyridyl-S-triazine (TPTZ,99 %), Linalool, Trolox (97%) are come under AR grade, Methanol (MeOH, 99.85%) GC grade and Quercetin (95%) HPLC grade.

Sample collection: Fresh leaves of *G. quaesita* and *G. zeylanica* were plucked (Sample size-03) around Matara, Sri Lanka which were authenticated in Peradeniya botanical garden, Sri Lanka**.** Healthy leaves were washed with tap water followed by distilled water and open-air dried for 3 days. Dried leaves were ground into powder by using grinder to be used in the extraction process.

Extraction: Two extraction techniques; maceration and sonication were used to optimize the extraction method to extract important phytochemicals. Ground powder (20.00 g) was macerated with MeOH (300 ml) for 48 hours at room temperature (RT) (Tachakittirungrod *et al*., 2007) and other part of ground powder (20.00 g) was sonicated by using ultrasound-assisted-extractor (ROCKER Ultrasonic cleaner, Model: SONER 202H) with MeOH (200 ml) for one hour at RT (Musa *et al*., 2011, Nguyen *et al*., 2013). The extracts were filtered through cotton plug and after concentration at 45^0C using a rotary evaporator (HAHNVAPOR, Model No: HS-2005S) crude extracts were obtained and stored at 4 ^{0}C till use.

Phytochemical qualitative analysis: Screening tests for phytochemicals namely alkaloids, glycosides, flavonoids, saponins, tannins, terpenoids, carbohydrates, phenol, phlobatannins, protein, coumarins, anthocyanins, chalcones, phytosterol, soluble starch, anthracene derivatives, anthraquinones, betacyanin and quinones were carried out in triplicates for the each methanolic extracts of leaves of two varieties, following the standard procedures described in the literatures with slight changes (Wadood *et al*., 2013,Gayathri *et al*., 2014).

Phytochemical quantitative analysis: The ground powder of methanolic extract (0.10 g) was dissolved in small amount of DMSO and diluted with methanol (100 ml) to make 1000 ppm concentration and used for quantification of phenolics, tannins, flavonoids and terpenoids using spectrophotometric methods.

Total Phenolic contents (TPC) and Total Tannin contents (TTC): The TPC and TTC were determined by Folin and Ciocalteu method which is described in the literature (Abeysuriya *et al*., 2020, Ekwueme *et al*., 2015, Prabhavathi*et al*., 2016). TPC is expressed in Gallic acid equivalents (mg GAE/g of methanolic extract) while TTC is expressed in Tannic acid equivalents (mg TAE/g of methanolic extract).

Total Flavonoid contents (TFC): The TFC was determined by spectrophotometric method with $AlCl_3$ described by Pêkal *et al* (2014) and Fernandes *et al.* (2014).The TFC is expressed in Quercetin equivalents (mg QE/g of methanolic extract).

Terpenoid contents (TC): The TC was determined by spectrophotometric method with phosphomolybdic acid (Ekwueme *et al*., 2015).TheTC is expressed in Linalool equivalents (mg LE/g of methanolic extract).

Antioxidant analysis

DPPH radical scavenging assay: The free radical scavenging activity of methanolic extracts was determined by the method discriped by Mosquera *et al.* (2009) with some slight changes. The DPPH solution in methanol (0.06 mM, 3.0 mL) was mixed with 1.5 mL of methanolic extract at different concentrations. The samples were kept in the dark for 30 minutes, and absorbance was measured at 517 nm (Ascorbic acid was used as the standard).

Ferric Reducing Antioxidant Power (FRAP) Assay: The ferric reducing power of the methanoilic extracts were determined using a standard method described in the literarure (Firuzi *et al*., 2005, Gliszczyñska-Świgło, 2006, Biglari *et al*., 2008). About 3 ml of freshly prepared FRAP reagent (300 mM acetate buffer-pH 3.6: 10 mM TPTZ in 40 mM HCl: 20 mM $FeCl_3$ in 10 : 1 : 1 ratio) was mixed with 100 µL of diluted sample and absorbance at 593nm was recorded after 30 min incubation at 37 °C. Trolox (0–400 ppm) was used for calibration.

Proximate analysis: Standard protocols of Association of Official Analytical Chemists (AOAC) were followed for the estimation of proximate composition such as moisture, ash, crude fiber, crude fat, crude protien, carbohydrate, total solids and energy (Nielsen 2010, Ilodibia *et al*., 2014, Maisarah *et al*., 2014)

Statistical analysis: Different statistics such as analysis of variance (ANOVA), T-test (LSD) (LSD-Least Significant Difference) and Non-parametric Cochran's Q test were carried out for analyzing the data obtained and comparisions. SAS, R-studio and Excel were used to perform the statistical analysis. Data were reported as means ± standard deviation.

RESULTS AND DISCUSSION

Extraction: An efficient extraction method is necessary to get almost all phytochemicals seperated from the plant.Out of many methods, solvent extraction techniques have been widely used. In this study, two different solvent extraction techniques, *i.e.;*maceration (conventional) and ultrasound-assisted-extraction (non-conventional) were compared to obtain extract rich in bioactive compounds. Comparison has been evaluated by using extraction yield and phytochemical qualitative analysis. The Fig.1 indicates about the comparison of both macerated and sonicated extraction yields. Sonicated extraction yields are comparatively higher in both garcinia varieties than macerated one while yield of *G. quaesita* has shown the highest percentage in both macerated and sonicated extraction.

Phytochemical qualitative analysis: The phytochemicals in the methanolic extracts of garcinia varieties is tabulated in Table 1. It showed the presence of highly important secondary metabolites in the leaves of both garcinia varieties. Out of them, flavonoids, saponins, alkaloids, glycosides, phenol, tannins, terpenoids, phytosterol, carbohydrates, proteins, soluble starch, coumarins, anthracene derivatives, betacyanin, anthraquinones and quinones are remarkable in the leaves of both garcinia varieties under two different extraction methods while some phytochemicals such as anthocyanins, phlobatanins and chalcones are absent in methanolic extracts.

Non-parametric Cochran's Q test was analyzed to make sure the presence and the absence of the phytochemical's availability in each plant samples statistically. Non-parametric Cochran's Q test has proofed that both *G. quaesita* and *G. zeylanica* leaves methanolic extracts contain these revealed phytochemicals and there is no significant

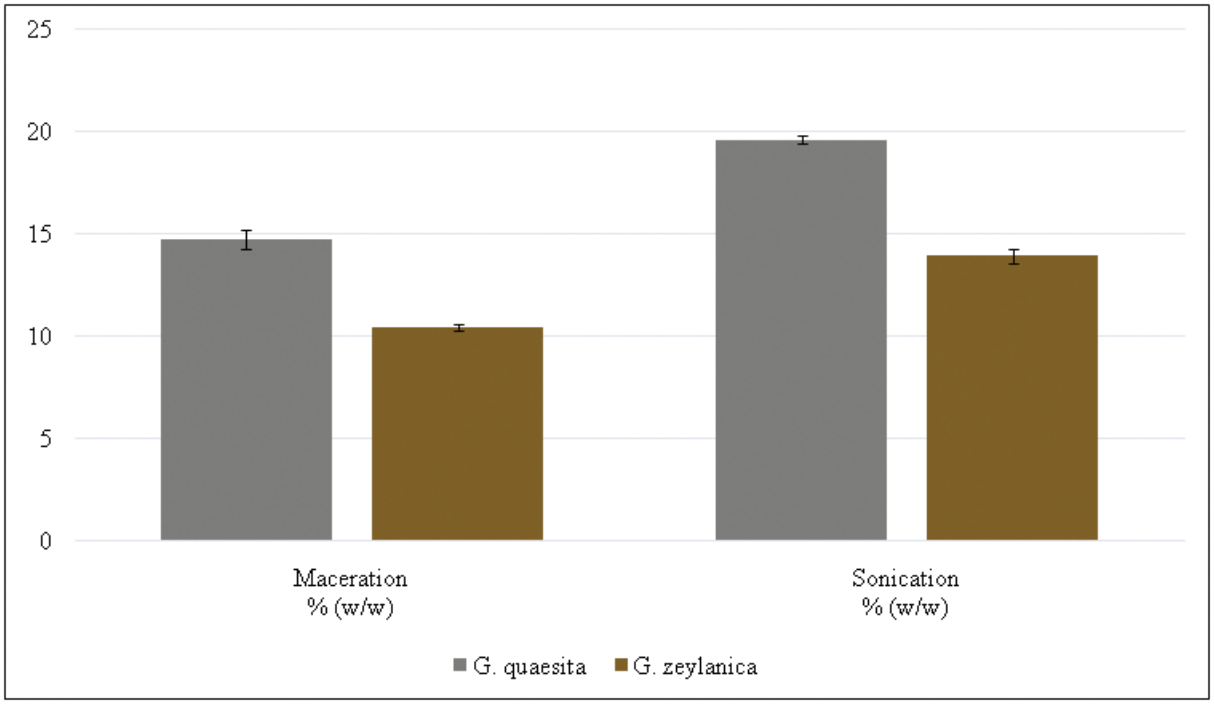

Fig. 1: Comparison of extraction yield by maceration and sonication of two garcinia varieties

Table 1: Statistically analyzed phytochemical screening results of methanolic extracts of leaves of two garcinia varieties under two extraction conditions i.e., sonication (S) and maceration (M).P: Present, A: Absent, 1: *G. quaesita*, 2: *G. zeylanica*.

Phytochemicals	Test method	1		2	
		M	S	M	S
Alkaloids	1). Mayer's Test	P	P	P	P
	2). Wagner's Test	P	P	P	P
	3). Dragendroff's Test	P	P	P	P
Glycosides	1). Keller-kilani Test	P	P	P	P
	2). Modified Borntrager's Test	A	A	A	A
	3). Legal's Test	P	P	P	P
Flavonoids	1). Alkaline reagent Test	P	P	P	P
	2). Shinoda Test/ Mg turning Test	P	A	P	A
	3). Lead acetate Test	P	P	P	P
	4). $AlCl_3$ Test	P	P	P	P
	5). NH_4OH Test	P	P	P	P
Saponins	1). Froth Test	P	P	P	P
	2). Olive Oil Test	P	P	P	P
Tannins	1). Bramer's Test	P	P	P	P
	2). Lead Acetate Test	P	P	P	P
Terpenoids	1). Salkowski's Test	P	P	P	P
	2). Liebermann- Burchardt Test	P	P	P	P
	3). Copper acetate Test	P	P	P	P
Carbohydrate	1). Fehling's Test	P	P	P	P
	2). Benedict's Test	P	P	P	P
	3). Molisch's Test	P	P	P	P
Phenols	1). Ferric Chloride Test	P	P	P	P
Phlobatannins	1). HCl Test	A	A	A	A
Protein	1). Xanthoproteic Test	P	P	P	P
	2). Biuret Test	A	A	A	A
	3). Ninhydrin Test	P	P	P	P
Coumarins	1). UV light Test	A	A	A	A
	2). NaOH Test	P	P	P	P
Anthocyanins	1). HCl & NH3 Test	A	A	A	A
Chalcones	1). NaOH Test	A	A	A	A
Phytosterol	1). Salkowski's Test	P	P	P	P
Soluble Starch	1). KOH Test	P	P	P	P
Anthracene Derivatives	1). Chloroform Test	P	P	P	P
Anthraquinones	1). Borntrager's Test	P	P	P	P
	2). Borntrager's Test 02	P	P	P	P
Betacyanin	1). NaOH Test	P	P	P	P
Quinones	1). H_2SO_4 Test	P	P	P	P

difference in presence chemicals constituents in both macerated and sonicated methanolic extracts of both varieties.

According to the yield percentage, ultrasound-assisted-extraction has shown the highest values in both garcinia varieties whereas phytochemical qualitative analysis has revealed that there is no

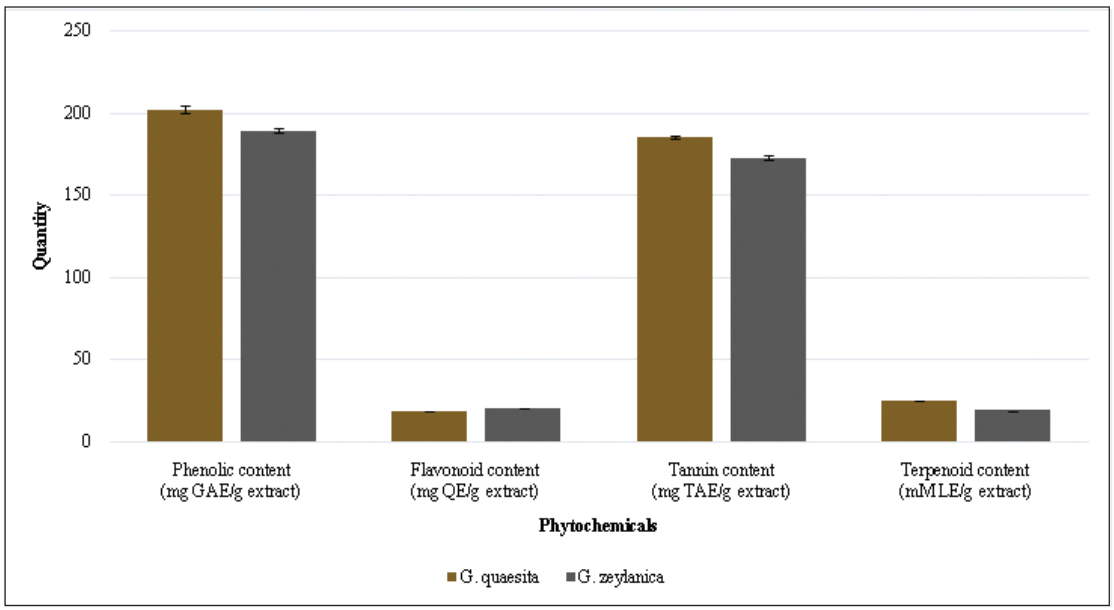

Fig. 2: comparison of phytochemical quantitative analysis data for macerated methanolic extracts of two garcinia varieties.

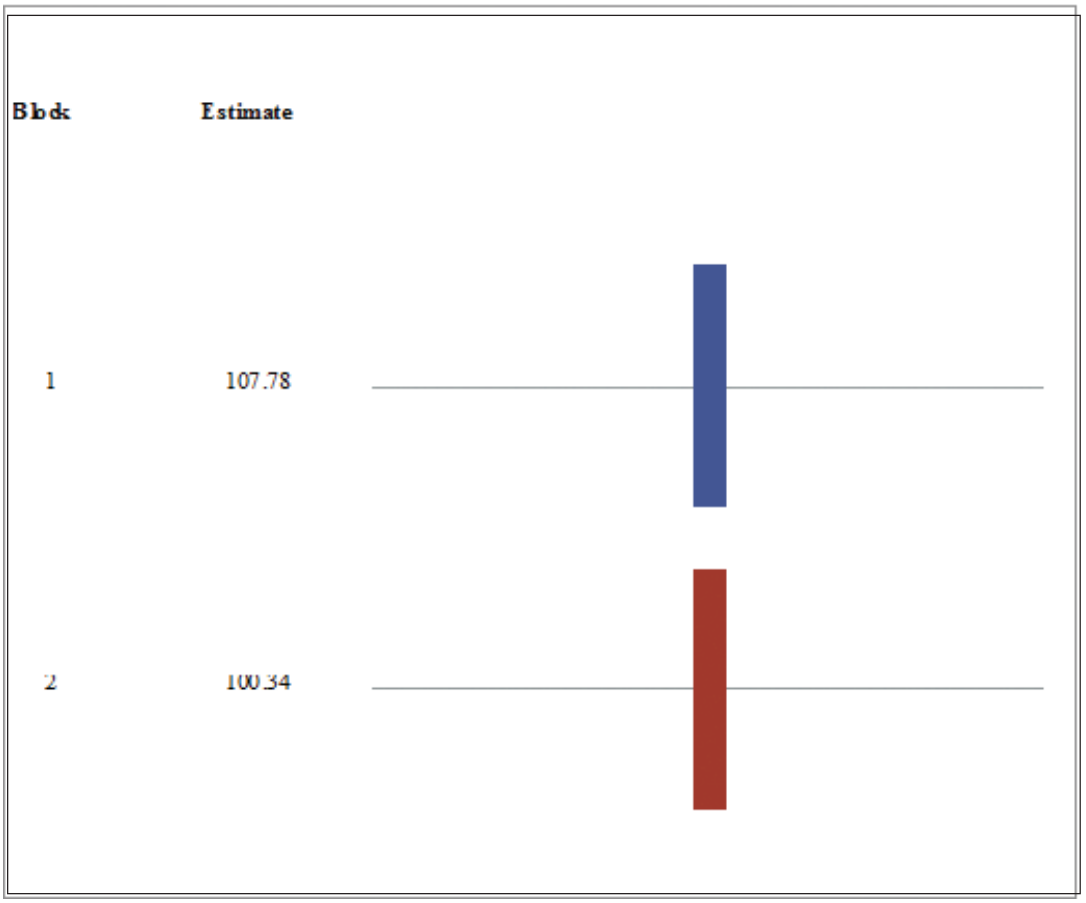

Fig. 3:T-test (LSD) for overall phytochemicals quantification of two garcinia varieties (Alpha = 0.05) (1: *G. quaesita*, 2: *G. zeylanica*, means covered by the same bar are not significantly different).

significant difference in presence of compounds in both macerated and sonicated methanolic extracts of two varieties. As sonication time is shorter (1 hour) than maceration (48 hours) and the consumption of solvent in sonication is also less than maceration, sonication would be recommended to extract active chemical constituents. This result supports the outstanding pharmacological activities associated with garcinia, which have been reported by various researchers and the applications of garcinia in traditional medicine. Even though, qualitative phytochemical data of both garcinia varieties revealed no significant differences between them, raising a question whether there will be a significant difference in the quantity of them.

Phytochemical quantitative analysis

Quantitative analysis of some important phytochemicals such as polyphenol, tannin, flavonoid, and terpenoid in the leaves indicated that both *G. quaesita* and *G. zeylanica* contain in varying amounts and the data is represented in Fig. 2. Interestingly, polyphenolic, tannin and terpenoid contents were high in *G. quaesita*; 202.14 ± 2.27 mg GAE/g extract, 185.45 ± 0.86 mg TAE/g extract and 25.23 ± 0.03 mM LE/g extract, respectively. Contrastingly, flavonoid content was high in *G. zeylanica* (20.29 ± 0.10 mg QE/g extract).

Total phytochemicals quantity was statistically analyzed by using T-test (LSD) and which is shown in Fig. 3. The statistical analysis clearly concludes that there is a significant difference in between both garcinia varieties and interestingly *G. quaesita* shows the highest phytochemical contents.This phytochemical quantification strongly revealed about the importance of each variety of garcinia, and the statistical analysis indicated that the uniqueness of both garcinia varieties in phytochemical quantity.

Antioxidant analysis

DPPH and FRAP assays were used. The results of DPPH assay are expressed in IC_{50} values while the results of FRAP assay are expressed in mg Trolox Equivalents/g extract and the results of both assays have been presented in Table 2.

Table 2: Antioxidant analysis data of methanolic leaf extracts of two garcinia varieties. Values represent mean ± standard deviation of triplicate sample (TE: Trolox Equivalents)

Antioxidant Assay	*G. quaesita*	*G. zeylanica*	Ascorbic acid
FRAP assay (mg TE/g extract)	225.63 ± 2.01	154.91 ± 6.96	N/A
DPPH assay IC_{50} value (ppm)	47.45 ± 0.03	39.59 ± 0.02	4.06 ± 0.01

According to the results of radical scavenging assay, the highest radical scavenging activity was observed in *G. zeylanica*. The statistical analysis with T-test (LSD) of DPPH assay perfectly revealed that both *G. quaesita* and *G. zeylanica* are statistically significance while *G. zeylanica* has shown the highest radical scavenging activity out of selected a garcinia variety which is shown in Fig.4. As phytochemical qualitative and quantitative analysis have revealed about the availability of vast array of bioactive compound present in both *G. quaesita* and *G. zeylanica* methanolic leaves extracts, further studies needed to reveal the pharmacological properties of these two Sri Lankan endemic species.

According to the FRAP assay, both *G. quaesita* and *G. zeylanica* extracts had reducing power but not at the same level. The result clearly indicated that the macerated methanolic extract of *G. quaesita* leaves had higher reducing power than *G. zeylanica*. Fig. 5 explains about the statistical analysis of FRAP assay for garcinia varieties. According to the T-test (LSD), FRAP assay also clearly revealed that there are significant differences between the varieties and interestingly, FRAP assay clearly revealed *G. quaesita* having the highest potential of reducing power.

Proximate analysis

Table 3 presents the proximate composition of leaves of selected garcinia varieties. Moisture, total solid, ash, fat, fiber, protein and carbohydrate contents were recorded in percentage of dry material whereas energy was recorded in kcal/100 g. According to the T-test (LSD) statistic, there are variation between the varieties.

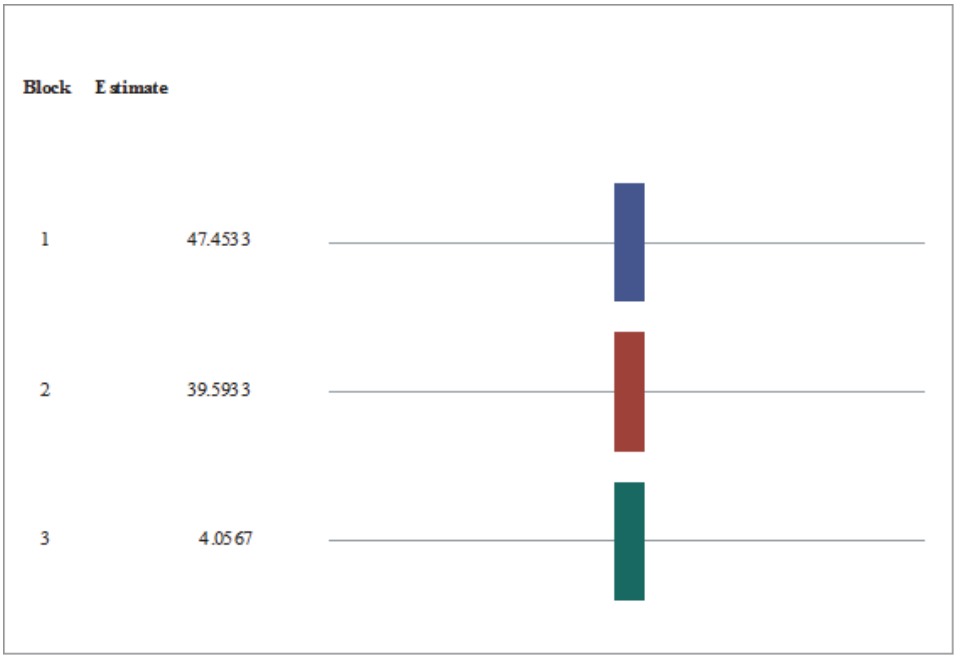

Fig. 4: T-test (LSD) for DPPH radical scavenging activity assay of methanolic extract of two garcinia varieties (Alpha = 0.05) (1: *G. quaesita*, 2: *G. zeylanica*, 3: Ascorbic acid, means covered by the same bar are not significantly different).

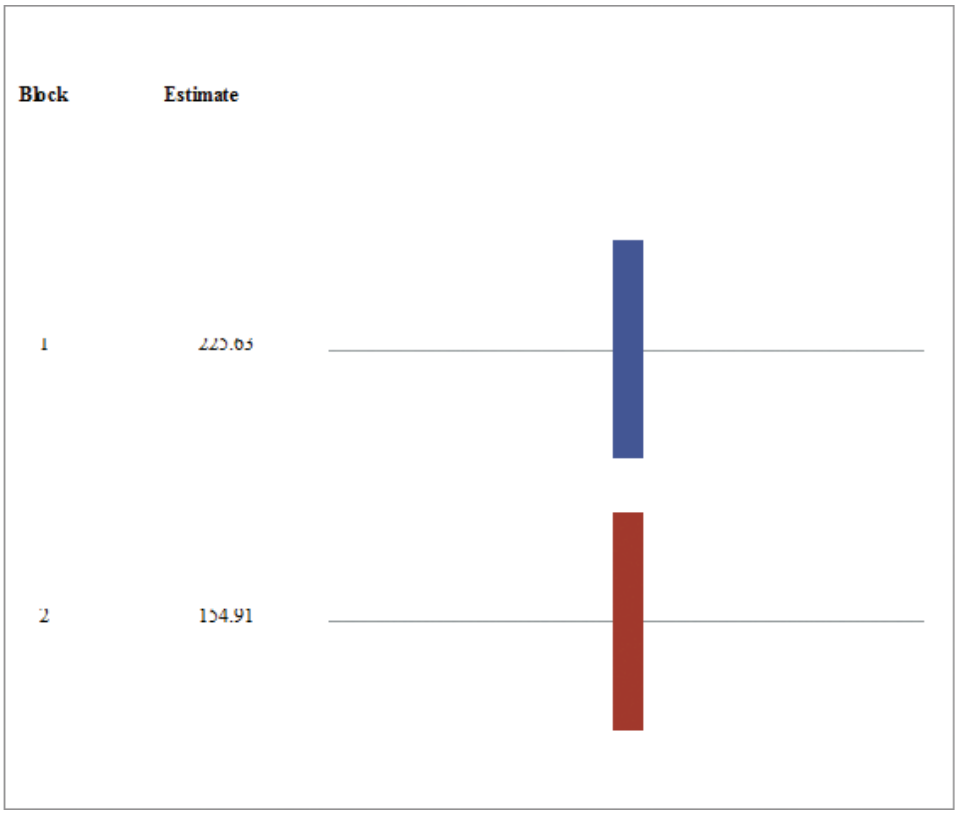

Fig. 5: T-test (LSD) for FRAP analysis of methanolic extract of two garcinia varieties (Alpha = 0.05)(1: *G. quaesita*, 2: *G. zeylanica,* means covered by the same bar are not significantly different).

Table 3: Proximate analysis data of dried leaves of two garcinia varieties. Values represent mean ± standard deviation of triplicate sample

Plant Name	Moisture (%)	Total solid (%)	Ash (%)	Crude fat (%)	Crude fibre (%)	Crude protein (%)	Carbohydrate (%)	Energy (kcal/100g)
G. quaesita	17.32 ± 0.03	82.68 ± 0.03	4.95 ± 0.60	2.47 ± 0.13	27.83 ± 1.08	8.75 ± 0.00	66.51 ± 0.74	323.24 ± 1.82
G. zeylanica	22.16 ± 0.06	77.84 ± 0.06	4.39 ± 0.10	2.34 ± 0.22	26.23 ± 0.05	9.64 ± 0.14	61.47 ± 0.30	305.49 ± 0.85

According to the T-test (LSD) statistic, moisture content was significantly different between two varieties yet highest was observed in *G. zeylanica*. In contrast to moisture content the highest total solid percentage was observed in *G. quaesita* whereas ash, fat and fiber contents were significantly no differences between two varieties. Protein and carbohydrate contents of garcinia leaves revealed that there is significant difference between the two varieties and protein content of *G. zeylanica* was relatively higher than *G. quaesita*. In contrast, carbohydrate content was higher in *G. quaesita* than *G. zeylanica*. Finally, energy level in garcinia leaves have shown significant differences each other but out of both varieties *G. quaesita* has revealed the highest energy level. Therefore, this proximate analysis of garcinia leaves clearly indicated that each variety having proximate composition at different levels.

CONCLUSION

This is the first detailed study on phytochemical, proximate composition, and antioxidant properties of leaves of two Sri Lankan endemic Garcinia varieties *viz*., *G. quaesita* and *G. zeylanica*. All the varieties contain diverse of pharmacologically important bioactive chemical compositions but no significant difference in phytochemical profile of leaves among both garcinia varieties selected in this study. Moreover ultrasound-assisted extraction, an accelerated extraction method would be recommended for extraction of bioactive compounds efficiently. Both garcinia varieties show antioxidant capacity and *G. quaesita* is placed at the top. Proximate composition reveals each variety has unique characteristic in most of the parameters. Therefore, this study will be a repository for the scientific community to be used in further studies and give overview on suitability on value addition via functional foods and nutraceuticals to be used in health promotion aspects.

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Kiwifruit (*Actinidia deliciosa*) propagation: Influence of stock plant sexuality on root development

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ABSTRACT

Kiwifruit (Actinidia chinensis) is a dioecious vine, commercially propagated through hard wood stem cuttings. To evaluate the effect of sexuality of donor plants on rooting of stem cuttings of kiwifruit and the effect of different concentrations of Indole butyric acid (IBA), a study was undertaken with factorial arrangement and completely randomized design including four commercial varieties and 6 replications. The IBA hormonal treatments were applied at four levels i.e. 0, 250, 500 and 1000 mg/l. Three female varieties known as Abbott, Hayward, Hayward Behi and one male genotype (pollinizer) were evaluated. Morphological parameters including largest and smallest roots, root numbers, leaf area and rooting percentage of the stem cuttings were recorded during three months. The biochemical parameters such as total carbohydrates, phenols, chlorophylls and endogenous Indole-3- acetic acid (IAA) content of donor plants were also estimated. Analysis of variance showed that auxin treatment had positive effect at many parameters as compared to control. The parameters like smallest root length, root numbers and rooting percentage were positively affected by hormonal treatment. Application of IBA @ 500 mg/l showed maximum length (0.766 cm) and the highest number of roots (0.5). Among varieties, the Hayward and male genotype showed maximum root length as well as root numbers. Rooting percentage of male genotypes was found to be the highest (28.35 %) as compared to female varieties. Among hormonal treatments IBA 1000 mg/l led to highest percentage of rooting (21.67 %). The highest chlorophyll rate (3.49 mg/g) and greatest total sugar content (0.011 mg/g) were recorded in Abbott variety stock plants. The mean leaf area (1501 mm^2) of stock plants was maximum in Hayward. The estimation of endogenous Indole Acetic Acid (IAA) by HPLC showed that the pollinizer (male) had maximum amount (1.052 mg/l) of this natural hormone. The correlation among traits revealed that a positive correlation between root numbers, rooting percentage and callus size with the content of endogenous IAA may be observed. The results definitely proved that the sexuality of mother plant has effective role in rooting and the male genotype has higher capacity to root development.

Keywords: Kiwifruit, propagation, IBA, IAA, Rooting, Sexuality

INTRODUCTION

The kiwifruit, often shortened to kiwi in many parts of the world (*Actinidia deliciosa*), is gaining popularity worldwide due to its rich nutritional, medicinal values with phytochemicals properties as it contents a lots of bioactivities, such as antitumor, anti-inflammatory, antioxidant, hypoglycemic, and hypolipidemic activities (Khan *et al.,* 2019). The flowers are fragrant, dioecious or bisexual, borne singly or in triple in the leaf axils, are 5- to 6-petalled, white at first, changing to buff-yellow, 2.5-5 cm broad, and both sexes have central tufts of many stamens though those of the female flowers with no viable pollen. The flowers also lack nectar. It flowers in November. Male and female flowers appear on different plants (dioecious) and both sexes have to be planted in close proximity for fruit set (Shastri *et al.*, 2012). Currently 85% of the kiwifruit produced from *Actinidia deliciosa* and the remaining 15% of *Actinidia chinensis* (Ferguson, 1990).

The main kiwifruit female commercial cultivars are Abbott, Manti, Alison, Brono and male cultivars Matoa and Tomori. Italy is now the leading producer of kiwifruit in the world, followed by New Zealand, Chile, France, Greece, Japan and the United States (Shastri *et al.*, 2012). Kiwifruit is usually propagated through cuttings. Though it can be propagated from seed but seedlings are not recommended because of its dioecious nature as the sex is unknown until flowers are produced. Propagation of kiwifruit with different types of stem cuttings such as hardwood, semi hardwood and soft wood cuttings have been successfully implemented,

but the technique and rooting ability are different. However, IBA increased rooting of all three kinds of cuttings (Hartman *et al.,* 2002). It is established fact that to harness the maximum efficiency from a crop cultivation, use of genuine planting material is the foremost requirement. Due to heterozygous nature of fruit crops, use of vegetatively propagated planting material is the scientific practices (Ghosh and Brea, 2015). Numerous factors such as genetic, environmental and chemical factors are involved in successful rooting of cuttings (Macdonald, 1993; Sweta, 2005). The IBA is the best and most popular commercial growth regulator to accelerate the rooting of cuttings (Hartman *et al.,* 2002). Various methods of IBA application were already tried *viz*., direct use of powder, rapid dipping method and hormonal solution, however, the sexuality of mother plant and its impact of root initiation was not previously studied. The present research work was undertaken to ascertain the possibility of cutting propagation of four different varieties (three female varieties as compared to one male (pollinizer) genotype. Furthermore, the role of IBA pre-treatments and the IAA content of stock plants and its possible correlation with rooting capability of cuttings were studied.

MATERIALS AND METHODS

The present study was undertaken in Gorgan University of Agricultural Sciences and Natural Resources, Iran. The experiment was laid out as complete randomized design with six replications. Three female varieties known as Abbott, Hayward, Hayward Behi and one male genotype (pollinizer) were evaluated for their capacity to root development. Thirty hard wood cuttings from each variety as well as male genotype were procured from a commercial kiwifruit orchard in December. These were shortened to 25-30 cm (at least four nodes) and their basal parts were dipped in different concentrations of IBA (0, 250, 500 and 1000 ppm) for ten seconds prior to insertion in rooting substrates. These were then inserted in a temporary medium comprising perlite and sawdust (2:1) for three weeks in which bud sprouting was occurred. Due to utilization of mature canes as source of cuttings, the flower bunches also were produced on some sprouted new shoots those were also manually removed. The cuttings were then transferred to medium comprising washed sands under mist system (Fig. 1). Misting was scheduled for two minutes spray in every 40 minutes to maintain average relative humidity of greenhouse in 70%±5. Air temperature and light intensity fluctuation on the bench surface of greenhouse were 25-35°C and 11,000-14,000 lux during mid-day, respectively. In order to prevent fungal infection a general recommended fungicide (Carbendazim, 0.2% w/v) was also sprayed every 20 days on to the bench surface. Morphological parameters of rooted cuttings including root numbers, largest and smallest roots and rooting percentage were measured 90 days after inoculation. Furthermore, total sugars, mean leaf area and endogenous level of Indole-3- acetic acid (IAA) of donor plants were also measured. The amount of total sugars was estimated using anthrone reagent method (Thimmaiah, 2004). The High performance liquid chromatography (HPLC) was utilized for estimation of endogenous IAA. The IAA standard was dissolved in methanol (HPLC grade) in a range of 0–9 mg/l. Reproducibility of the results was confirmed by the analysis of three injections per each sample. The retention time was recorded to be 7 min for both standard and samples. Analyses were performed in a HPLC unit (Merck-Hitachi, Germany–Japan) comprising a L-7100 HPLC pump equipped with a photodiode array L-2450 detector (Hitachi, Japan) and software EZ chrome. Separation was carried out using an Exsil C18 cartridge (250 × 4.6 mm). The mobile phase was an isocratic of deionized water–acetonitrile (73%, 26% respectively (pH=4)) at a flow-rate of 1 ml/min and the detection wavelength of 280 nm. All samples were run in triplicate. The linearity range was determined from 1–9 mg/l with a 20 µl injection volume (R2 = 0.997). Sample aliquots were filtered through a 0.20 µm nylon membrane (ALBET® LabScience, Germany) prior to injection.

The data was analyzed using SAS software, based on completely randomized design with 6 replications. The mean comparison was performed with LSD test.

RESULTS AND DISCUSSION

In the present study, the exogenous IBA application had positive effect of adventitious root

Table 1. Analysis of variance of measured rooting parameters of stem cuttings of four kiwifruit genotypes

Sources of variation	df	Mean root length	No. of roots	Rooting percentage
Treatment	3	4.91*	1.57*	1262.34*
Genotype	3	28.93**	23.47**	114.61**
Genotype × treatment	9	3.28ns	3.25ns	118.55ns
Error	64	2.68	2.27	5.54
C.V.	-	30.04	29.62	6.98

*significantly different $p < 0.05$, **: are significantly different $p < 0.01$; ns: non-significant

Table 2. The influence of IBA treatments and genotypes on rooting parameters of kiwifruit hardwood cuttings.

	IBA treatment (mg/l)				Kiwifruit cultivars			
Trait/Treatment	0	250	500	1000	Abbot	Hayward	H. Behi	Male
Rooting (%)	15.85c	12.51d	16.68b	21.67a	0.84d	25.01b	12.51c	28.35a
No. of roots	0.441b	0.440b	0.500a	0.683a	0.008b	0.816a	0.291b	0.95a

* Means in the same row followed by different letters are significantly different at $P < 0.01$ using LSD-Test.

Table 3. : The average leaf area, total sugars and endogenous level of Indole Acetic Acid (IAA) measured in stock plants of different kiwifruit cultivars.

cultivar	Average leaf areacm²	Total sugarsF.W. mg/g	Endogenous Indole Acetic Acid (IAA)
Abbot	1464b	0.011a	0.298d
Hayward	1501a	0.002b	0.667c
Hayward Behi	1292c	0.0018b	1.052b
Male	1246d	0.0018b	1.398a

* Means in the same column followed by different letters are significantly different at $P < 0.01$ using LSD-Test.

Table 4. The correlations among measured parameters of stock plants and cutting response.

Rooting	No. of roots	Callus size	IAA	Leaf area	Total sugars	Traits
				-	1	Total sugars
				1	0.486ns	Leaf area
			1	-0.890**	-0.789**	IAA
		1	0.427ns	-0.047*	-0.639*	Callus size
	1	0.829**	0.661*	-0.268ns	-0.761**	No. of roots
1	0.992**	0.372ns	0.711**	-0.320ns	-0.836**	Rooting

induction in kiwifruit hard wood stem cuttings. The analysis of variance of measured rooting parameters of stem cuttings of four kiwifruit genotypes is shown in Table 1. It is clear that the role of treatment and/or genotype is statistically significant. Among different concentrations, 1000 mg//l IBA induced highest number of roots but it has no statistically significant difference with 500 mg/l. The highest rooting percentage was also recorded in IBA 1000 mg/l (Table 2). The role of auxin on root induction was already reported in numerous studies with different plant species (Hartman *et al*., 2002; Bartolini *et al*., 2008). Tanimotto (2005) in a critical review article, has stated the classical views of root-growth regulation by auxin and gibberellin

The results clearly demonstrated that male genotype has superior ability to induce adventitious roots. The male genotype had highest rooting

Fig 1: The kiwifruit hardwood cuttings under mist system 4 weeks after planting (left), root emergence on a stem cutting treated with 1000 mg/l IBA two months after planting (right).

percentage as well as number of roots as compared to three female varieties (Table 2). The effect of genotype on rooting have been reported by other researchers in different plant species. For example, during research on the effect of genotype on micropropagation of walnut trees, rooting between the two genotypes was observed differed that one reason was the plant hormone levels and peroxidase activity (Scaltsoyiannes *et al*., 1998). The male kiwifruits are more vigorous than female vines. As they do not produce fruit, the photosynthates are utilized for their vegetative growth. Furthermore, in the present study, the estimation of endogenous level of IAA by HPLC revealed that the pollinizer (male) genotype had significantly higher concentration (1.052 mg/l) of this natural hormone. The endogenous IAA level estimated in male vines, showed statistically significant difference as compared with other three female varieties (Table 3). It may be stated that such IAA level was definitely effective in root development on cuttings procured from mother plants. The role of endogenous Auxins on root formation was already reviewed by Blakesley *et al*. (1991). It has already reported that Auxin can be synthesized in young leaves and cotyledons (Ljung *et al*. 2001). The same long-distance pathway that carries carbohydrates from "source" to "sink" also facilitates the bulk flow of auxin and other hormones such as ABA and cytokinins as well as mRNA and proteins (Robert and Friml, 2009). So, it was not unusual that cuttings prepared from this genotype had maximum rooting percentage. The correlation data (Table 4) also clearly showed that the endogenous IAA was positively correlated with rooting percentage. The Abbott donor plant had lowest concentration of IAA and it could be one of the reasons for poor root ability of cuttings prepared from this variety (Table 2).

The correlations among measured parameters of stock plants and their cutting response have been shown in Table 4. In the present study, the rooting parameters were recorded 90 days after inoculation. However, the cutting basal ends form callus tissues just 3 weeks following inoculation. The callus tissue formation had significant positive correlation with number of roots. The endogenous level of IAA was also positively and statistically correlated with rooting percentage and number of roots.

According the results, it may be concluded that IBA treatment had really positive effect on adventitious root induction on kiwifruit hardwood cuttings. The stem cuttings treated with 1000 mg/l IBA showed maximum root length and rooting percentage (Fig 1). Hence, the same treatment may be suggested for commercial nurseries involved in kiwifruit propagation. On the other hand, the results showed that male vines would have higher rooting potential as compared to female ones. Furthermore, the endogenous IAA content of male stocks was significantly higher than female varieties and this parameter was positively correlated with rooting parameters.

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Genetic variation for morphological and physico-chemical traits in jamun (*Syzygium cuminii* Skeels)

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ABSTRACT

Among 23 genotypes of jamun studied, the maximum fruit weight (15.67 g), fruit diameter (2.68 cm) and pulp weight (11.83 g) were recorded in the type KJS-300. The genotype KJS-18 recorded significantly the longest fruit (3.90 cm), while the shortest (2.05 cm) was recorded in KJS-43. The highest pulp content (80.64%) was recorded in KJS-25. The maximum pulp to seed ratio (6.17) was recorded in KJS-02 and the lowest seed weight (1.17 g) was recorded in the KJS-24. The highest TSS (21.23%) and acidity (0.66%) was recorded in genotype KJS-03 and KJS-25 respectively. Significantly, maximum TSS to acid ratio (73.75) was recorded in KJS-300. The maximum anthocyanin (1.36 OD) and ascorbic acid (28.17 mg/100 g) were recorded in KJS-18 and KJS-02 respectively. The highest total sugar (16.37%) and non-reducing sugar (16.36%) were registered in the genotype KJS-09.The maximum sugar to acid ratio (53.24) and reducing sugar (0.030%) was recorded in the genotype KJS-26 and KJS-43 respectively. Results of study revealed that the genotypes KJS-85, KJS-4, KJS-9, KJS-1, KJS-3, KJS-300, KJS-18, KJS-12, KJS-2, KJS-25 and KJS-43 were found to be promising and could be used for further evaluation.

Keywords: Jamun genotypes, variations, physical and chemical parameters

INTRODUCTION

The jamun (*Syzygium cuminii* Skeels), a member of family Myrtaceae is one of the important underutilized fruits, widely distributed throughout tropic and subtropics as stray plantation or avenue. It is native to India or East Indies. In India, the maximum number of jamun trees is found scattered throughout the tropical and subtropical regions. It has gained tremendous importance and recognition in recent past not only because of its hardy nature but also for its uncomparable medicinal and nutritional properties. Besides, using for dessert purpose, fruits are used for preparation of delicious beverages, jellies, jam, squash, wine, vinegar, etc. (Singh *et al.,* 210). Jamun is highly cross-pollinated crop; hence wide variability is common in this species though it is propagated through nucellar seedlings. It offers more avenues for establishing desirable clones by simple seedling selection. The elite trees selected for total yield, size and quality of fruit, etc., are the starting point of any fruit tree improvement activity. They also provide scion material for vegetative propagation which can straightway go as improved planting material (Anon., 1989). In nature, lot of variations with respect to fruit shape and size, fruit and pulp colour, TSS, acidity and earliness in bearing of this fruit are evident. So, advantage of these variations can be taken to evolve a selection of superior seedling. Considering the importance and potentiality of cultivating this crop, there is an urgent need for improvement and to develop varieties suitable for cultivation under different agro-climatic areas. Due to lack of any suitable/recommended variety, the farmers have been planting trees of either seedling origin or grafted plants of unknown yield potential and fruit quality. These trees show wide variation in their fruiting, yield and fruit quality. Therefore, a study was conducted to find out variation in physico-chemical characteristics of jamun and to identify superior clones and elite seedlings in northern part of Karnataka state.

MATERIALS AND METHODS

An experiment was conducted in the Department of Fruit Science, K.R.C. College of Horticulture, Arabhavi in Gokak taluk of Belgaum district, Karnataka during 2009 and 2010. The study was conducted in a randomized block design with three replications. In this present investigation, twenty-three promising genotypes were collected form Gokak (Kolavi, Dhupadhal, Bategeri, Saudatti, Kaitnal etc.). The fruits from those trees

were collected from May 2009 end to first fortnight of June 2009. Further these fruits were analyzed for morphological, yield, physic-chemical parameters. The extent of variations in tree morphology from different locations were classified with respect to approximate age *viz*., old (more than 40 yrs.), medium (20 to 40 yrs.) and young (less than 20 yrs.); shape of canopy *viz*., round and oval; tree height *viz*., large (more than 15 m), medium (10 to 15 m) and small (less than 10 m); spread; tree circumference and yield was also recorded in terms of kg per tree. Further, the extent of variations in fruit physico-chemical traits from different locations was estimated. Six fruits from each selected trees were randomly taken for measuring physical attributes like weight, length, diameter, length to diameter ratio, pulp weight, pulp content, pulp to seed ratio, seed weight, seed length, fruit volume, seed volume following standard procedures. Total soluble solids were estimated in terms of degree Brix with the help of Erma (0 to 32%) hand refrectometer. Titrable acidity was estimated by titrating 50 ml juice against 0.01 N NaOH solution using phenolphthalein as indicator (Anon, 1976). Reducing sugars and total sugars were determined as per the Dinitro salicylic acid method (Miller, 1972) and values obtained were expressed as percentage on fresh weight basis. The percentage of non-reducing sugars was obtained by subtracting the values of reducing sugar from that of total sugars. Anthocyanin was determined by using spectronic-20 spectrophotometer where the optical density values of fruit juice extracted with acetone solution were recorded at 350 nm. Ascorbic acid was estimated by dye method using 2,6-dichlorophenol indophenol modified procedure of AOAC (Anon., 1984) and was recorded in milligram per 100 gram fruit flesh.

RESULTS AND DISCUSSION

The data pertaining to morphological and yield attributes of jamun trees presented in (Table 1). Among the 23 genotypes, 7 genotypes were found to be old age of more than 40 years, 13 genotypes of medium age (20-40 years) and 3 genotypes were young aged having less than 20 years. Among the studied genotypes, 7 genotypes were found to be oval canopy, while 16 genotypes were found to be round canopy. The average spread of 23 genotypes was 9.08 m. The highest spread was recorded in KJS-09 (15.75 m), while minimum spread were recorded in KJS-21 (6.65 m), followed byJS-22 (6.65 m) and KJS-43 (6.65 m). One genotype was small statures, nine genotypes were medium and 13 genotypes were large statured. The mean circumference of the tree was 1.56 m. The maximum circumference was recorded in KJS-04 (2.8 m), while minimum circumferences were recorded in KJS-08 (1.2 m) followed by KJS-21 (1.2 m), KJS-22 (1.2 m) and KJS-43 (1.2 m). The maximum yield was recorded in KJS-85 (400 kg), while the lowest yield were recorded in the genotype KJS-21 (80 kg), KJS-22 (80 kg) and KJS-43 (80 kg). The mean yield of 23 genotypes was 130.87 kg. The data pertaining to physical and chemical quality attributes of jamun fruits showed significant differences except for pulp percentage and a high degree of variability for all the characters were studied (Table 2 and 3). The fruit weight varied from 4.00 g in KJS-43 to 15.67 g in KJS-300. Higher fruit weight is a preferred character in jamun. The average weight per fruit of KJS-18, KJS-01 and KJS-04 were on par with KJS-300. Fruit length was found maximum in KJS-18 followed by KJS-04. Fruit diameter was found highest in KJS-300. The minimum fruit length and diameter was recorded in KJS-43 genotype. Fruit length to diameter ratio is a measure of fruit shape. Higher length to diameter ratio indicated the cylindrical shape, while lower ratio suggested the oblong and round shape of the fruits. The maximum length to diameter ratio (1.70) was recorded in KJS-18, which exhibited cylindrical fruits. The minimum fruit length to diameter ratio was measured in KJS-43, which exhibited fruits towards the round shape. Variation in jamun genotypes with above characters was earlier reported from Goa (Devi *et al*., 2002), Karnataka (Inamdar *et al*., 2002, Prabhuraj *et al*., 2003 and Swamy *et al*., 2017), West Bengal (Kundu *et al*., 2001) and Gujarat (Singh *et al*., 2019a and 2019b).

Pulp weight, seed weight, seed length, pulp to seed ratio, volume of fruit and volume of seed also varied significantly except for pulp content (Table 2). The maximum pulp weight was recorded in KJS-300 while minimum was recorded in KJS-43. The highest pulp content was recorded in KJS-25, followed by KJS-09 and KJS-04 genotypes. The

Table 1: Tree morphological characters and yield of different genotypes of jamun

Genotype	Age	Shape of canopy	Spread (m)	Height	Circumference (m)	Fruit Yield (Kg/ tree)
KJS01	Old	Round	10.90	Large	1.50	200
KJS02	Old	Round	9.40	Large	1.30	150
KJS03	Old	Round	10.14	Large	2.10	200
KJS04	Old	Round	12.75	Large	2.80	150
KJS07	Old	Round	10.65	Large	1.80	100
KJS08	Old	Round	8.80	Large	1.20	100
KJS09	Old	Oval	15.75	Large	2.10	150
KJS11	Medium	Oval	8.75	Large	1.60	120
KJS12	Young	Oval	8.65	Large	1.50	100
KJS14	Young	Oval	9.60	Large	1.50	100
KJS18	Medium	Oval	8.10	Medium	1.60	150
KJS21	Medium	Round	6.65	Medium	1.20	80
KJS22	Medium	Round	6.65	Medium	1.20	80
KJS23	Medium	Round	7.50	Small	1.40	100
KJS24	Medium	Round	9.25	Medium	1.40	100
KJS25	Medium	Round	10.15	Medium	1.70	100
KJS26	Medium	Oval	7.50	Large	1.40	100
KJS27	Young	Oval	6.82	Large	1.40	100
KJS85	Medium	Round	6.85	Large	1.40	400
KJS43	Medium	Round	6.65	Medium	1.20	80
KJS95	Medium	Round	9.25	Medium	1.40	100
KJS96	Medium	Round	10.15	Medium	1.70	100
KJS300	Medium	Round	8.10	Medium	1.60	150

lowest pulp content was recorded in KJS-24. Though the maximum weight of the fruit was recorded in KJS-300 with 75.49% pulp content, the maximum pulp content was observed in KJS-25, which weighed only 10.33 g. This may be because of the rudimentary seed with almost negligible seed weight in KJS-25. Seed weight in various genotypes ranged from 1.17 g in KJS-24 to 3.63 g in KJS-12. Lower seed weight is a preferred character for table purpose jamun. Seed length varies from 1.52 cm in KJS-43 to 3.02 cm in KJS-85. The above observations revealed that while selecting a superior jamun genotype, pulp content should be given more importance rather than the fruit weight (Devi *et al*., 2002). The pulp to seed ratio in various genotypes ranged from 2.30 in KJS-43 to 6.17 in KJS-02 and showed wide range of variability. Similar results were also reported from Goa and Gujarat (Devi *et al*., 2002, Singh *et al.,* 2007b, 2010) and Karnataka (Prabhuraj *et al*., 2003, Inamdar *et al*., 2002). Higher pulp to seed ratio is a desirable character for table purpose jamun and for breeding quality fruits. Therefore, one should select pollen parent as genotype having high fruit pulp to seed ratio.Volume of fruit and seed was recorded maximum in KJS-300, while minimum was found to be in KJS-43.

The data presented in Table 3 reveals wide variation in chemical composition of the fruits of all the 23 genotypes. TSS content varied from 10.40% in KJS-21 to 21.23% in KJS-03. The highest anthocyanin was recorded in genotype KJS-18 while minimum was recorded in KJS-01 and the mean value for this character was 0.65 OD. The mean acidity was 0.48 per cent and the lowest acidity was recorded in KJS-95, KJS-96, KJS-300, which was on par with KJS-26, KJS-43 and KJS-85. The highest acidity was recorded in genotype KJS-25. Ascorbic acid content was highest in KJS-02 and lowest in KJS-12. The highest total sugar content was registered in KJS-09 which was on par with KJS-01 and the lowest was noticed in KJS-

Table 2: Physical character of jamun fruit in different genotypes

Genotype	Fruit weight (g)	Fruit length (cm)	Fruit diameter (cm)	Length: diameter	Pulp weight (g)	Pulp (%)	Seed weight (g)	Seed length (cm)	Pulp: Seed	Fruit volume (ml)	Seed volume (ml)
KJS01	13.67	3.40	2.37	1.44	10.67	77.98	1.87	2.44	5.93	12.97	2.68
KJS02	11.67	3.52	2.16	1.62	8.67	74.20	1.67	2.44	6.17	10.08	1.10
KJS03	10.83	3.43	2.19	1.57	8.50	78.48	1.83	2.45	4.25	10.25	1.87
KJS04	13.50	3.67	2.38	1.55	10.75	79.63	2.00	2.45	5.37	11.08	2.27
KJS07	9.33	3.20	2.10	1.52	7.00	75.02	2.42	2.19	3.02	9.12	1.38
KJS08	9.67	3.18	2.20	1.44	7.33	75.80	2.00	2.34	5.00	9.67	1.14
KJS09	8.50	3.07	2.05	1.50	6.83	80.35	2.00	2.18	3.42	8.25	1.42
KJS11	10.83	3.38	2.18	1.55	7.50	69.25	2.00	2.29	3.75	10.80	1.34
KJS12	11.67	3.61	2.20	1.65	9.00	77.12	3.63	2.47	3.28	9.92	1.35
KJS14	11.33	3.31	2.29	1.44	8.33	73.52	2.63	2.35	3.56	10.40	1.42
KJS18	14.00	3.90	2.30	1.70	10.67	76.14	2.17	2.41	4.97	11.25	1.88
KJS21	10.50	3.58	2.16	1.65	7.83	74.57	1.83	2.39	4.50	9.92	1.79
KJS22	10.33	3.58	2.15	1.66	7.92	76.67	2.00	2.48	5.89	9.55	2.10
KJS23	11.17	2.97	2.49	1.19	7.33	65.62	2.50	2.22	3.37	10.27	2.32
KJS24	6.33	2.42	1.83	1.32	3.67	57.82	1.17	1.91	3.39	5.17	1.35
KJS25	10.33	3.39	2.11	1.61	8.33	80.64	1.83	2.33	4.75	10.55	1.32
KJS26	11.50	3.41	2.25	1.51	9.00	78.26	1.83	2.34	5.08	10.75	1.92
KJS27	9.83	3.38	2.08	1.63	7.67	77.92	1.50	2.21	5.58	10.00	1.90
KJS85	7.80	3.20	2.10	1.52	5.54	71.02	2.31	3.02	2.40	7.62	1.64
KJS43	4.00	2.05	1.80	1.13	2.67	66.50	1.29	1.52	2.30	3.22	0.52
KJS95	9.67	2.99	2.19	1.36	7.25	74.87	2.42	2.13	3.18	10.38	2.14
KJS96	9.67	3.00	2.22	1.35	7.29	75.39	2.38	2.59	3.40	9.55	2.55
KJS300	15.67	3.42	2.68	1.27	11.83	75.49	2.83	2.42	4.46	15.00	3.25
Mean	**10.51**	**3.26**	**2.19**	**1.49**	**7.89**	**74.45**	**2.09**	**2.33**	**4.22**	**9.82**	**1.77**
S.Em±	**0.93**	**0.10**	**0.06**	**0.04**	**0.75**	**7.79**	**0.38**	**0.07**	**0.64**	**0.41**	**0.07**
CD (5%)	**2.66**	**0.29**	**0.17**	**0.13**	**2.15**	**NS**	**1.07**	**0.20**	**1.83**	**1.18**	**0.20**
CV (%)	**15.38**	**5.43**	**4.81**	**5.20**	**16.56**	**17.96**	**31.29**	**5.36**	**26.38**	**7.30**	**7.06**

NS = Non-significant

Table 3: Chemical parameters of fruits in different genotypes of jamun

Genotype	TSS (%)	Anthocyanin (OD Value)	Acidity (%)	Ascorbic acid (mg/100g)	Total sugar (%)	Reducing sugar (%)	Non-reducing sugar (%)	Sugar to Acid ratio	TSS to Acid ratio
KJS01	18.00	0.17	0.57	24.31	16.147	0.021	16.13	28.53	31.99
KJS02	12.50	0.27	0.51	28.17	14.271	0.018	14.25	27.99	24.50
KJS03	21.23	0.35	0.54	21.34	14.911	0.021	14.89	27.50	39.20
KJS04	14.80	0.43	0.42	23.44	11.402	0.012	11.39	27.41	35.88
KJS07	11.97	0.41	0.61	22.34	12.242	0.012	12.23	20.72	20.22
KJS08	11.47	0.42	0.54	20.43	13.845	0.011	13.83	26.05	21.77
KJS09	15.88	0.36	0.63	22.28	16.376	0.016	16.36	27.82	27.33
KJS11	14.40	0.60	0.56	23.40	12.312	0.015	12.30	22.19	25.96
KJS12	10.60	0.42	0.48	18.25	11.433	0.016	11.42	25.08	23.23
KJS14	13.63	0.93	0.59	19.43	15.546	0.009	15.54	26.39	23.15
KJS18	13.67	1.36	0.65	20.33	13.398	0.005	13.39	21.03	21.49
KJS21	10.40	0.59	0.58	23.47	14.437	0.014	14.42	25.09	18.11
KJS22	11.97	0.71	0.65	27.17	8.233	0.020	8.21	12.77	18.45
KJS23	15.00	0.22	0.53	24.26	13.611	0.014	13.60	25.94	28.44
KJS24	16.67	0.82	0.42	21.77	14.361	0.015	14.35	33.94	39.18
KJS25	19.83	0.85	0.66	26.47	10.268	0.011	10.26	15.66	30.25
KJS26	14.13	0.53	0.27	27.53	14.202	0.008	14.19	53.24	52.88
KJS27	14.00	0.65	0.48	24.55	9.640	0.013	9.63	20.14	29.16
KJS85	16.80	0.37	0.29	20.23	13.460	0.015	13.45	46.10	57.54
KJS43	11.57	0.85	0.31	22.20	9.124	0.030	9.09	29.60	37.55
KJS95	10.93	1.22	0.21	22.10	10.050	0.013	10.04	48.20	52.45
KJS96	10.90	1.04	0.21	22.11	10.089	0.012	10.08	47.15	50.94
KJS300	15.43	1.32	0.21	20.51	10.150	0.020	10.13	48.41	73.75
Mean	**14.16**	**0.65**	**0.48**	**22.87**	**12.59**	**0.015**	**12.57**	**29.87**	**34.06**
S.Em±	**0.686**	**0.021**	**0.038**	**0.267**	**0.550**	**0.003**	**0.550**	**2.132**	**2.670**
CD (5%)	**1.96**	**0.06**	**0.10**	**0.76**	**1.57**	**0.009**	**1.57**	**6.079**	**7.61**
CV (%)	**8.39**	**5.67**	**13.95**	**2.02**	**7.57**	**40.79**	**7.58**	**12.37**	**13.58**

22. KJS-43 recorded highest content of reducing sugar, while lowest was noticed in KJS-18. The maximum non-reducing sugar content was recorded in KJS-09, while the lowest was observed in KJS-22. KJS-26 was recorded to have the highest sugar to acid ratio and lowest was recorded in KJS-22. TSS to acid ratio ranged from 18.11 in KJS-21 to 73.75 in KJS-300 and the mean of this parameter was found to be 34.06.

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Antimitotic and genotoxic effect on the meristematic cells of *Allium cepa* L. of the alkaloid and flavonoid fractions of the leaves of *Peganum harmala* L. from the Laghouat region, Algeria

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ABSTRACT

Medicinal plants are an inexhaustible source of secondary metabolites, namely polyphenols including flavonoids, alkaloids and terpenoids, which generate various biological activities. Keeping this in mind, we were interested in a spontaneous plant Peganum harmala L. to demonstrate the antimitotic and genotoxic effect of the alkaloid and flavonoid fractions (aqueous and butanolic) of the leaves of this species on root meristematic cells of Allium cepa L. Mitotic indices, phase indices, aberration indices as well as cytotoxicity limit values were calculated for our different samples and controls, namely the negative (distilled water) and positive controls (colchicine 1mg/ml and quercetin 1mg/ml). The results indicate a mitodepressant and sublethal effect, observed with the alkaloid and flavonoid fractions of the leaves of Peganum harmala L. Exposure of meristematic cells to the samples resulted in an antimitotic and genotoxic effect translated into a large number of chromosomal, nuclear and cellular aberrations.

***Keywords*:** Alkaloid fraction, *Allium cepa* L., antimitotic, flavonoid fractions, genotoxic effect, *Peganum harmala* L.

INTRODUCTION

Man has always relied on plants for their vital needs. This interest has increased due to the therapeutic benefits of medicinal plants (Nandi and Ghosh, 2016; Sabitha Rani *et al.,* 2019). In order to contribute to the valorization of local medicinal plants known for their therapeutic virtues, we were interested in the present work in the study of the antimitotic and genotoxic activity of the alkaloid and flavonoid fractions of the leaves of *Peganum harmala* L., harvested in Timzerth, Dayate Aiat in the wilaya of Laghouat in southern Algeria. *Peganum harmala* L., local name Harmel, is an endemic species of the family Zygophyllaceae, it grows in semi-arid areas on sandy, stony and nitrate soils. This plant is known for its richness in various secondary metabolites mainly alkaloids, coumarins and flavonoids (Al yahya, 1986). It has anti-bacterial, anti-fungal, anti-viral, anti-oxidant, anti-diabetic, anti-tumor, anti-leishmaniasis, insecticidal effect, cytotoxic activity, as well as hepato-protective effects (Jinous and Fereshteh, 2012). However, if mis-used it can be very toxic for animals and humans in particular. It is responsible for paralysis of the nervous system and causes death by respiratory arrest in vertebrates, and can cause pregnancy termination in women (Bellakhdar, 1997).

The Allium test is a standardized test for monitoring cytogenotoxicity, known for its simplicity and reliability (Fiskesjö, 1985). It combines two targets: toxicity and genotoxicity. In addition, it is important to note that its cost is low and that it correlates well with mammalian test systems (Fiskesjö, 1985; Cabrera and Rodriguez 1999; Jovtchev *et al*., 2002; Grant 1994;1999; Yi and Meng 2002).The method for assessing chromosomal aberrations at the meristematic cell level of *Allium cepa* L. roots is validated by the International Programme on Chemical Safety, World Health Organization (IPCS, WHO) and the United Nations Environment Programme (UNEP) as an effective test for the analysis and monitoring of the genotoxicity of natural substances (Cabrera and Rodriguez, 1999). Therefore, we used the *Allium cepa* L. test to evaluate the antimitotic and genotoxic effect of our samples.

MATERIALS AND METHODS

Plant material

The leaves of *Peganum harmala* L., were freshly harvested from ten healthy individuals in April 2015. Sampling was carried out randomly in dayate Aiat, Timzerth region, wilaya of Laghouat (Algeria). Bioclimatically, the study area is located in the arid zone, with a dry season of 11 months per year (Limane *et al.,* 2014).The samples were placed in paper bags and stored in a cooler and then refrigerated until they were used in the laboratory. The identification of the plant was carried out by Professor Smail-Saadoun Noria, Director of the Natural Resources Laboratory (LRN), University Mouloud Maameri, Tizi-Ouzou (Algeria).The harvested leaves were dried in the shade at room temperature, lying on the laboratory bench for 10 days. They were ground to a fine powder, stored in smoked glass jars, sealed and stored in the laboratory cabinet.

Extraction of alkaloids

To extract the total alkaloids, we used the conventional method of Harbone (1998), which we have optimized. A quantity of 5g of the powder from the leaves of *Peganum harmala* L. was degreased in 10ml of petroleum ether for 24 hours at room temperature then filtered with Wattman paper N° 1 paper and the filtrate was discarded. The powder thus degreased was macerated in methanol under the same conditions as before. The filtrate thus recovered was dry evaporated at 60°C, then taken up by chloroform and acidified by 5% HCl at pH=3. The acidic aqueous phase was recovered, to which chloroform was added, then basified with 5% Na_2CO_3 at pH=9. The chloroform phase is then evaporated. The dry residue which represents the total alkaloids is taken up by chloroform which will be evaporated dry at 60°C and the residue thus obtained is recovered by distilled water.

Extraction of flavonoids

For the extraction of flavonoids, we used the liquid method of Bekkara *et al*. (1998) where 1g of vegetable powder is brought into contact with 20ml of methanol at room temperature. After 24 hours incubation, the solution is filtered with Wattman paper N° 1, the filtrate obtained is evaporated at 60°C, the resulting residue is solubilized with a mixture of distilled water and ethyl acetate and then decanted. The aqueous phase is recovered to which 10 ml of n-butanol is added. After decantation, two distinct phases are obtained: the flavonoic butanolic phase and the aqueous flavonoic phase. The solvents are evaporated and the residues obtained are solubilised in distilled water, stored at +6 °C and protected from light in smoked bottles.

The *Allium cepa* L. test

The plants used for the *Allium cepa* L. test are individuals belonging to the species *Allium cepa* L. (family Amaryllidaceae or Alliaceae, depending on the chosen taxonomic treatment), commonly known as onion. The *Allium cepa* L. tests genotoxicity using chromosomes (Bonciu *et al.,* 2018).

The protocol followed for the *Allium cepa* L. test is that of Fiskesjö (1985) with modifications made by Shweta *et al*. (2012). Bulbs of *Allium cepa* L. onions of the same size are placed in beakers containing water for 72 hours at laboratory room temperature. After elongation, the 0.5 to 1 cm long root apexes were brought into contact with the alkaloid fraction as well as the flavonoid fractions (aqueous and butanolic) of the leaves of *Peganum harmala* L. The negative control is represented by distilled water, moreover, the positive control is represented by colchicine (1mg/ml) for the alkaloid fraction and quercetin (1mg/ml) for the flavonoid fractions. After 24 hours incubationat ambient temperature, the roots were fixed in a freshly prepared mixture of one volume of acetic acid and three volumes of 95% ethanol (1:3 V/V). Fixation is intended to block any evolution of cell division and maintain the structural integrity of the chromosomes (Jahier, 1992).The roots thus fixed are colored by acetic carmine, prepared by boiling 55 ml of distilled water and 45 ml of acetic acid, add 0.5 g of carmine powder, keep boiling for 5 miutes. which is used for its double action as a fixative/stain and allows the observation of the cores. The treated root fragments were prepared between slides and lamellae. Cytogenenetic analysisis done with an optical microscope at magnification (×400).The blades are viewed from right to left and from top to bottom. The counting of the cells in mitotic division as well as the

anomalies generated by the action of our samples is performed on 350 cells of the different phases of mitosis, namely: Prophase (P), Metaphase (M), Anaphase (A) and Telophase (T).

Cytogenetic analysis is carried out by evaluating four biological parameters. For each sample, 5 tests were carried out (Fiskejo, 1993; Antosiewicz, *et al.,* 1990).The parameters evaluated were:

The mitotic index (MI): MI (%)= (Number of cells division / Number of cells examined (350 cells)) × 100

The limit value for cytotoxicity: LVC(%) = (MI of treated cells / MI of negative control cells) × 100

The phase index (PI): PI (%) = [(Number of cells in (P, M, A, T) /Number of cells examined (350 cells)] × 100

The aberration index (AI): AI (%) = [(Total chromosomal aberrations / Number of cells examined (350 cells)]× 100

Statistical analysis

ANOVA-type analyses of variance were carried out, in order to highlight significant differences between our extracts (alkaloid and flavonoid fraction) and the controls (negative and positive) used, with the STATISTICA Software at the threshold ($p<0.05$).

RESULTS AND DISCUSSION

Analysis of the meristematic cells of *Allium cepa* L. by the alkaloid fraction extracted from the leaves of *Peganum harmala* L. reveals a remarkable decrease in the mitotic index. The rate reached 36.60±1.47%, which is less than half the rate calculated for the negative control (92.20±1.34%). This index is close to the value of the mitotic index of cells treated with the positive control (colchicine) which is 35.00±1.16%, with the dominance of prophase whose phase index is equal to 11.94±6.87%. On the other hand, cells brought into contact with quercetin (positive flavonoid control), their mitotic index decreases to 44.40±0.72%, close to that obtained in the presence of the butanolic flavonoid fraction which reaches a value of 44.00±0.40%. On the other hand, for meristematic cells in contact with the aqueous flavonoid fraction, the mitotic index value is 62.60±0.82% (Fig.1). Meristematic cells treated with both the negative (distilled water) and positive controls (colchicine and quercetin), as well as our samples (alkaloid and flavonoid fractions) are mostly blocked in prophase. Note that the metaphase index is nil for the cells treated with the alkaloid and flavonoid fractions (Fig. 2).

The use of specific solvents of increasing polarity: methanol, ethyl acetate and n-butanol allowed the separation of flavonoids according to their degree of solubility in these extraction solvents and their structural complexity. Methanol removes non-phenolic compounds such as carotenoids, chlorophyll pigments and fats. In addition, ethyl acetate allows the extraction of monoglycosides. As for n-butanol, it allows the extraction of diglycosides and triglycosides (Sharaf *et al.*, 1997). According to Halim *et al*. (1995), the main flavonoids in the leaves of *Peganum harmala* are represented by: Acacetine-7-O-rhamnoside, Acacetine-7-O-[6 ‘’-O-glucosyl-2’’-O-(3’’’-acetylrhamnosyl)] glucoside and Acacetine-7-O-(2’’’-O-rhamnosyl-2 O-glucosylglucoside). *Peganum harmala* contains alkaloids of the type β-carbolines, the most important of which are harmine, harmaline, harmol and harmalol and quinazolines which are responsible for toxicological and pharmacological effects (Pulpati *et al.*, 2008; Beyer *et al*., 2009; Herraiz *et al*., 2010).

The standard ANOVA statistical analysis of mitotic indices revealed highly significant differences between the positive control (colchicine) and the negative control (P=0.00), between the negative control and the alkaloid fraction (P=0.00). Similarly, there were highly significant differences (p=0.00) between the mitotic index of the negative control, that of the positive control (quercetin) and that of the aqueous and butanolic flavonoid fraction. Highly significant differences were also observed between the mitotic index of the positive control (quercetin) and that of the aqueous flavonoic fraction. In addition, no significant difference (p=0.67) was observed between the mitotic index of the positive control (quercetin) and that of the butanolic flavonoic fraction. This confirms their action in inhibiting mitosis. However, non-significant differences were observed for both alkaloid and flavonoid fraction phase indices.

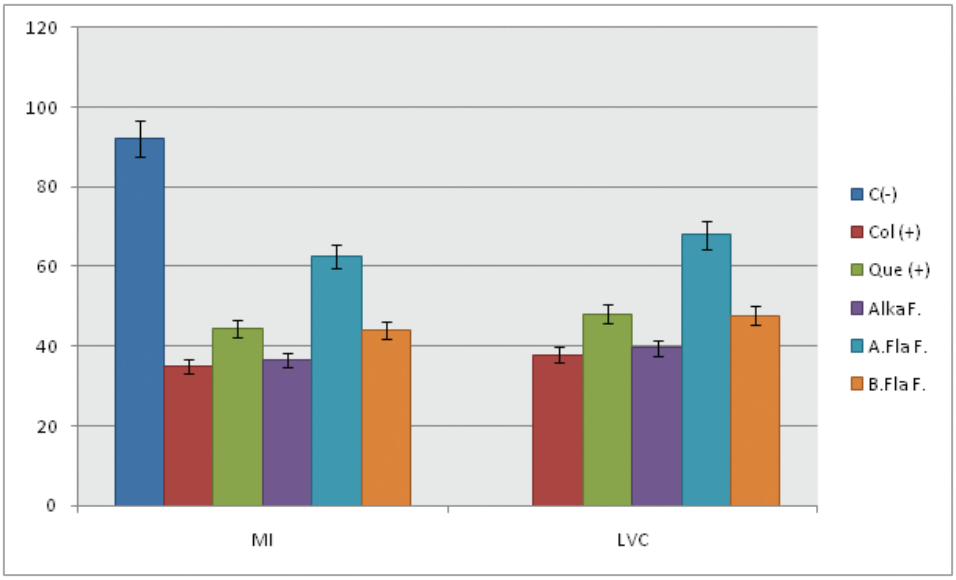

Fig. 1 : Mitotic index and limit value of cytotoxicity of the different fractions tested.
C(-): Negative control, Col(+): Colchicine (Positive alkaloid control), Que (+): Quercetin (Positive flavonoid control), Alka F.: Alkaloid Fraction, A.Fla F.: Aqueous Flavonoid Fraction, B.Fla F: Butanolic Flavonoid Fraction, MI: Mitotic Index, LVC: Limit Value for Cytotoxicity.

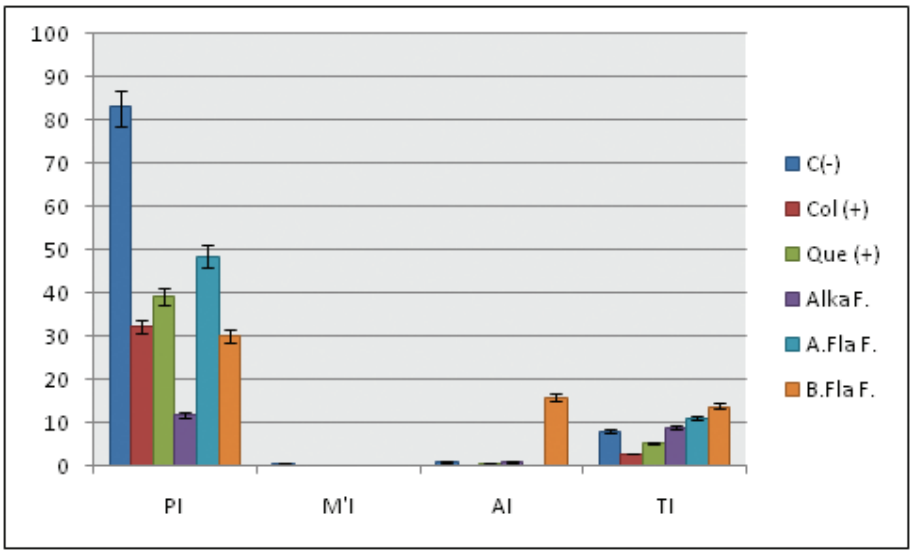

Fig. 2: Phase index of the different fractions tested.
PI: Prophase Index, M'I: Metaphase Index, AI: Anaphase Index, TI: Telophase Index.

The mitotic index is considered a parameter for estimating the frequency of cell division (Marcano *et al*., 2006). Decreased mitotic activity in root meristematic cells of *Allium cepa* L. indicates a mitosuppressive effect of the fraction and aqueous extracts of *Peganum harmala* L. Similar mitosuppressive effects were observed in meristematic cells of *Allium cepa* L. treated with aqueous extracts of five medicinal plants used in the Nigerian pharmacopoeia: *Azadirachta indica* A. JUSS; *Morinda ludica* Benth; *Cymbopogon citratus* DC. Stapf, *Mangifera indica* L. and *Carica papaya* L. at the following concentrations: 1, 2.5, 10, 20% (w/v) (Akinboro and Bakare, 2007). Several *in vitro* studies have shown the anticancer effect of flavonoids against many cancer cell lines as well as *in vivo*. Bosetti *et al*. (2005) and Fink *et al*. (2007) have shown that the consumption of

Table 1: Aberration indices assessed at the level of cells treated with positive controls and alkaloid and flavonoid fractions of *Peganum harmala* L. leaves.

Traitement	CA±SE	BC±SE	CE±SE	CB±SE	CF±SE	DEP±SE	GC±SE	CWN±SE	AB±SE	AC±SE	Total±SE
Col (+)	5.60±1.42	0.74±0.66	0.86±0.48	0.00±0.00	1.89±0.55	0.23±0.14	1.94±1.13	0.86±0.37	0.17±0.15	0.0±0,00	12.29±4.90
Que (+)	8.97±1.76	0.00±0.00	1.71±0.56	0.00±0.00	6.74±2.54	0.17±0.10	1.08±0.59	0.00±0.00	0.00±0.00	3.54±1.71	22.21±7.26
Alka F.	12.62±0.77	0.22±0.10	0.00±0.00	0.00±0.00	0.17±0.07	0.11±0.05	1.31±0.28	0.00±0.00	0.17±0.07	0.00±0.00	14.60±1.34
A. Fla F.	4.14±0.60	0.00±0.00	0.80± 0.54	0.06±0.04	0.85±0.34	0.00±0.00	0.00±0.00	0.62±0.56	0.11±0.09	0.00±0.00	6.58±2.20
B. Fla F.	10.57±0.67	0.51±0.25	4.40±2.16	0.00±0.00	3.94±1.75	0.17±0.01	0.34±0.03	0.68±0.06	0.06±0.02	7.31±1.39	27.97±7.28

C(-): Negative control, Col(+): Colchicine (Positive alkaloid control), Que (+): Quercetin (Positive flavonoid control), Alka F.: Alkaloid Fraction, A.Fla F.: Aqueous Flavonoid Fraction, B.Fla F: Butanolic Flavonoid Fraction.

SE: standard error; CA: chromosomal agglutinations; BC: binucleated cells; CE: cell elongations; CB: chromosomal bridges; CF: chromosomal fragmentations; DEP: Disorganization of the equatorial plate; GC: gigantic cells; CWN: cells without a nucleus (ghost cells); AB: apoptotic bodies; AC: absence of cytodieresis.Aberration ± Standard error (%).

flavonoids reduces the risk and incidence of several types of cancers, namely breast and lung cancer. The reduction in mitotic activity could be due to inhibition of DNA and nucleoprotein synthesis in the biological system (Chauhan *et al*., 1998) and modification or alteration in the expression of certain genes (Siddiqui *et al*., 2007; Sultan and Celik, 2009). Sadaf *et al.* (2021) demonstrated the anticancer effect of methanolic extracts of *Peganum harmala* L. seeds and roots on prostate cancer cell lines (PC3) as well as breast cancer cell line (MCF7). These significant antitumour and cytotoxic effects could be due to the presence of phytochemicals, including flavonoids and phenolic compounds.

The increase in the number of prophases in *A. cepa* cells treated with the aqueous extracts and leaf fraction of *Peganum harmala* L., suggests, according to Damato (1954), that this is due either to too long a duration of treatment or to the use of too high doses, resulting in slower entry into the other stages of mitosis, notably, metaphase, anaphase and telophase. Firbas and Amon (2014) noted that both anaphase, telophase and metaphase assays are suitable for the detection of genotoxic effects of ionizing radiation. Overall, the test of *Allium cepa* is proved to be a very convenient, highly sensitive and informative cytogenetic tool for rapid screening of ionizing radiation and radionuclide pollution (Firbas and Amon, 2017).

Fig. 1 also shows the limit value for cytotoxicity (LVC %) of meristematic cells of *Allium cepa* L. treated with the alkaloid fraction and flavonoid fraction of the leaves of *Peganum harmala* L. compared to positive controls. The ANOVA standard statistical analysis showed significant differences between the limit value for cytotoxicity of cells treated with the alkaloid fraction and those treated with the flavonoid fractions ($p=0.03$). On the other hand, no significant difference was observed between the limit value of cytotoxicity of quercetin and the butanolic flavonoid fraction ($p=0.76$).

When the mitotic index decreases below 22% of the control it causes what is called the "lethal effect" on the test organisms (Antosiewicz, 1990). A decrease in the mitotic index of 50% relative to the control is usually a sublethal effect (Panda and

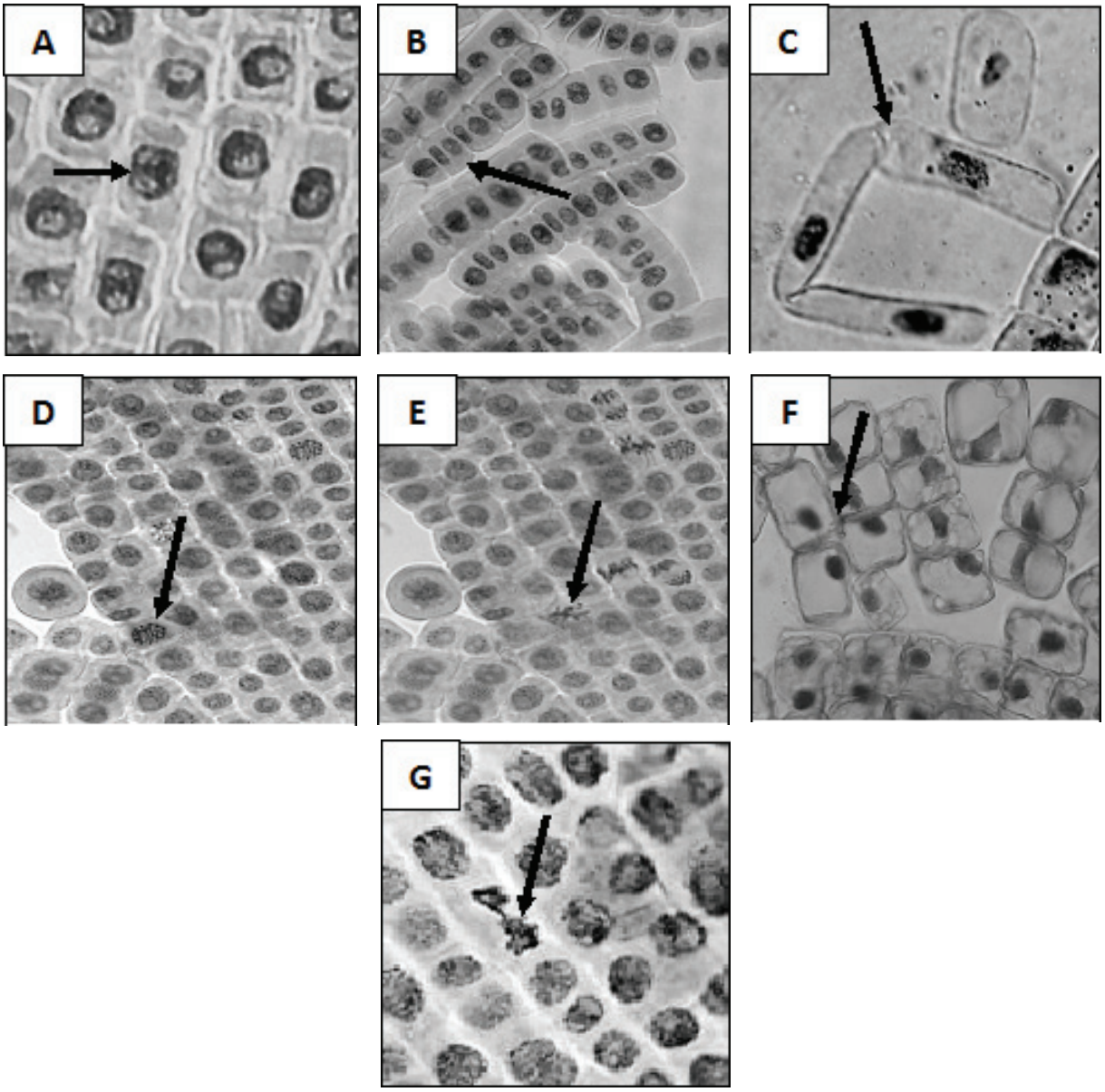

Fig. 3: the different aberrations observed in the meristematic cells of *Allium cepa* L., seen under an optical microscope (X400).
A: chromosomal agglutinations. B: binucleated cells, C: cell elongations.
D: chromosomal fragmentations. E: disorganization of the equatorial plate in metaphase. F: gigantic cells.
G: apoptotic bodies.

Sahu, 1985) and is referred to as the limit value for cytotoxicity (Sharma, 1983). According to these two definitions it can be deduced that the alkaloid fraction and the flavonoic butanolic fraction of the leaves of *Peganum harmala* L. are considered to be sublethal for the meristematic cells of *Allium cepa* L. in the same way as colchicine and quercetin.

Cytogenetic analysis of meristematic cells of *Allium cepa* L. treated with the alkaloid fraction and flavonoid fraction of *Peganum harmala* L. leaves indicates the presence of different types of cell division abnormalities. These abnormalities are expressed by varying levels of aberration indices relative to the positive controls (colchicine and quercetin) (Table 1). The main abnormalities observed are shown in Fig.3 and are as follows: Chromosomal agglutinations (Fig. 3A), binucleated cells (Fig.3B), cell elongations (Fig.3C),

chromosomal fragmentations (Fig.3D), disorganization of the equatorial plate in metaphase (Fig.3E), gigantic cells (Fig.3F) and apoptotic bodies (Fig.3G).

The statistical analysis ANOVA showed non-significant differences between the aberration indices for our different extracts, reflecting a similar genotoxic effect of our extracts with colchicine and quercetin (positive controls).

Chromosomal aberrations are changes in the structure of chromosomes resulting from the breakage or exchange of chromosomal material (Tülay and Özlem, 2010; Firbas and Amon, 2014). These observed aberrations indicate the effect of the aqueous extract and alkaloid fraction of the leaves of *Peganum harmala* L. on the organization of chromatin. This may be related to disorders in the quantity of histones, or other proteins, responsible for controlling the structure of nuclear chromatin (Stryer, 1997). Chromatin condensation can be produced as a result of stress conditions (Fusconi *et al*., 2006).

The frequent appearance of chromosomal agglutination in *Allium cepa* L. cells is probably due to an inhibition of the entry of prophases and a blockage of those in progress (Deysson, 1956). The presence of chromosomal bridges is a complex phenomenon related to fragmentation, stickiness of chromosomes, breaks and reunion of broken ends (Kabarity *et al*., 1974). Chromosomal fragmentations thus indicate the clastogenic potential of certain chemical compounds from plants; they can also be a consequence of anaphase/ telophase bridges (Chuhan and Gupta, 2005). Their action on chromosomes is generally localized at the DNA level (Chuhan and Sandararaman, 1990). Dose-response analyses are worth pursuing as they reflect the shift in genotoxicity from a simple yes/ no binary characteristic to a quantitative measure that can better inform risk assessments, since the margin of exposure and other toxicological principles can be taken into account (Dearfield, 2017; Dertinger, 2019).

CONCLUSION

Our work on the study of the antimitotic and genotoxic effect of the alkaloid and flavonoid fractions of the leaves of *Peganum harmala* L. on the meristematic cells of *Allium cepa* L. showed the accumulation of cells at the prophase stage, the presence of chromosomal fragmentation and agglutination, a cytotoxic effect on the cells resulting in gigantic cells, and the absence of cytodieresis. A mitodepressive effect on the root meristematic cells of *Allium cepa* L., was also observed in cells treated with the alkaloid fraction and those of flavonoids from the leaves of *Peganum harmala* L. considering them sublethal on the cells. The effect of *Peganum harmala* L. leaf extracts on other eukaryotic systems and by other experimental approaches should be investigated to obtain additional information on the antimitotic and genotoxic effect of the secondary metabolites of these extracts, which should contribute to the elucidation of their mechanisms of action.

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Vegetative propagation of *Acalypha hispida* through cuttings with different types of media

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ABSTRACT

Acalypha hispida is a wild flowering herbaceous plant commonly called as Red-hot cat's tail or Monkey tail, belongs to family Euphorbiaceae. It is commonly propagated by stem cuttings, but successful propagation of Acalypha hispida by stem cutting is harder, and even the basic information on these aspects are limited. Hence, an experiment was conducted to determine the effects of stem cutting types and growing media on the successful propagation of Acalypha hispida. Four types of stem cuttings (shoot tip, soft wood, semi hardwood and hardwood) and three types of media (pure sand, pure coir dust and 1:1 mixture of sand: coir dust v:v) were used, and the experiment was laid out in Complete Randomized Design (CRD) with 3×4 factor factorial arrangement. Data was analyzed by SAS 9.1.3 software. It was observed that there was a significant (P>0.05) interaction between the cutting types and media used in all the tested parameters except survival rate. All the type of cuttings planted in sand with coir dust showed highest percentage of rooted shoots with well formed root system. Hardwood cuttings planted in pure coir dust and 1:1 ratio of sand: coir dust showed not significant virtuous root weight (0.15g, 0.16g), number of leaves (13.10, 13.43), and shoot length (3.53 cm , 3.34 cm). Cutting types showed significant (P>0.05) in survival and hardwood cuttings showed highest survival rate (80.56%) compared to others. Accordingly, it could be concluded that hardwood cutting as the best material planted in a 1:1 mixture of sand with coir dust and pure coir dust medium can be used to propagate Acalypha hispida plants successfully.

Keywords: *Acalypha hispida*, media, propagation, rooting, stem cutting

INTRODUCTION

Acalypha hispida, the chenille plant, red hot cat's tail, monkey tail and fox tail are the names given around the world. It is a flowering shrub is a genus of the family Euphorbiaceae. It is mainly cultivated as an ornamental plant due to its attractiveness and brilliantly colored, furry flowers. Further, the leaves of the plants are laxative, diuretic in nature and used in the treatment of leprosy and gonorrhea. Various part of the plant is also used in infectious diarrhoea, pulmonary problems, and as an expectorant in asthma (Seebaluck *et al.,* 2015). The leaves of *Acalypha hispida* has been reported to have cytotoxic, antibacterial (Bokshi *et al*., 2012) antileprotic (McLaughlin *et al*., 1998), antimicrobial (Adesina *et al*., 2000) and antifungal (Ejechi and Souzey, 1999) properties.

Stem cutting is the most common material used for vegetative propagation of many plant species from herbaceous to woody plants, including ornamental plants. The success of propagation via stem cuttings is often affected by many aspects, including the status of the mother plant, type of growing medium, nature of cutting, rooting hormones and environmental conditions. A better medium which can be used for plant propagation is consisted of factors that offer optimumporosity, drainage and moisture retention.The requirements for a propagation mediumare somewhat different from a potting medium, which may have to sustain a growing plant for a prolonged period of time.The role of a propagation medium is to give support and moisture while the plant is developing. Generally, in propagation, growers use different types of planting media such as peat, perlite, vermiculite, sand and various organic and inorganic composted materials to prepare nutritious mixtures for plant propagation. Coir dust is a by-products available in locally from coconut manufacturing processing units, and are used as a propagation and

growing medium for propagation of several plants including medicinal plants such as *Momordica dioica* Roxb (Nawarathna *et al.,* 2020), *Salacia reticulata* (Nayana *et al.,* 2015), etc. for its better water holding capacity, higher contents of some nutrients such as potassium, sodium and magnesium (Abad *et al.,*2002).

Since there is lack of information available on propagation of *Acalypha hispida* plants, it is important to identify the proper successful and simple propagation technique for the efficient production of planting materials. Hence the present investigation was carried out with objectives of identification of most suitable planting material, growing media, and simple and effective protocol for in vivo propagation of *Acalypha hispida* plant.

MATERIALS AND METHODS

The study was conducted at the University of Colombo Institute for Agro-technology and Rural Sciences, Weligatta, Hambantota, Sri Lanka. The area falls under the low country dry zone agro-ecological region in Sri Lanka where the mean annual rain fall is 1250mm-1500mm and mean annual temperature ranging between 29^0C-33^0C. Common soil type representing area is reddish brown earth. Three types of growing media (pure coir dust, pure sand, 1:1 mixture of sand and coir dust parts by volume) and four types of stem sectionsas hardwood (fully matured brownish and woody parts), semi hardwood (partially matured and slightly woody), softwood (made up of soft and succulent parts just below the shoot tip) and shoot tips (topmost three nodes with shoot tip) were used for the experiment. The experiment was laid out in the Complete Randomized Design with factorial arrangement having twelve treatments and four replications. Each replication contained three experimental units.

A pest free, healthy mother plant was selected nearby the experimental area for collection of planting materials. Required amount of stem cuttings were collected from a single mother plant. Healthy branches of *Acalypha hispida* were detached from mother plant early in the morning and adaxial ends were dipped in water to prevent from drying and avoiding trapping of air bubble in vascular system until transport to the experimental area.

Pure sand and coir dust were sieved to remove unwanted materials and to get fine particles to facilitate rooting. Pots of 10cm× 15cm were prepared with black polyethylene and holes at the bottom to facilitate the drainage of excess water.The pots were filled with the potting mixtures and treated with a fungicide to sterilize the media. Nodal cuttings with different maturity stages were separated using sharp knife to prevent the tissue damages. A slant cut was made at the adaxial end of the each cutting just below the node to increase the surface area. The cut surface was garnished with a rooting hormone containing 0.03% Indole Butyric Acid to promote the rooting. Each cutting was carefully planted in polyethylene pots inserting at least a node completely to be inside the media.

Planted cuttings were maintained under completely sealed propagator covered using 500 gauge transparent polyethylene sheet. The structure was maintained under 50% shade condition throughout four weeks of time. Watering was not practiced during the period under propagator. After four weeks, cuttings were taken out from propagator and survival percentage, percentage of rooted cuttings, number of leaves, number of new shoots, shoot length, root number and root weight were measured.

The recorded data were statistically analyzed using PROC GLM procedures in SAS 9.1.3 statistical software. The difference between the treatments means were compared using Duncan Multiple Range Test (DMRT) at 5% significant level.

RESULTS AND DISCUSSION

Effect of cutting type and potting media on survival

There was no significant ($p<0.05$) interaction between the tested factors on survival of *Acalypha hispida* plants. Stem cutting types showed highly significant ($P>0.05$) effect on percentage survival. As shown in Table 1, among the four types of stem cutting, hardwood showed highest (80.60%) survival followed by semi hardwood, softwood and shoot tip with 55.60%, 38.90% and 19.40% respectively. The results suggested that matured

Table 1: Effects of cutting type and media on survival rate of *Acalypha hispida*

Treatments		survival rate
Media types	Sand	39.50[a]
	Coir dust	47.80[a]
	Sand + Coir dust	58.50[a]
	Significant	NS
Cutting types	Shoot tip	19.40[c]
	Softwood	38.90[bc]
	Semi hardwood	55.60[b]
	Hardwood	80.60[a]
	Significant	*

Means followed by the different superscripts in a same column are significantly different at DMRT 5%.
* Represents significant at 5% and ns represents not significant.

stem cutting of *Acalypha hispida* has been reliable in survival possibly due to the content of adequate food reserves which feed cuttings until it forms roots. This results were in agreement with observations on previous studies, where cuttings with larger diameter and longer length showed better survival rate and growth under normal conditions (Vigl and Rewald, 2014 on Salicaceae; Ouyang *et al.,* 2015). Furthermore, a study on cutting propagation of *Hevea brasiliensis* by Corpuz (2013) proved that, there was a significantly higher survival rate found in brown cut stem (hardwood) than green cut stems (softwood).

Effect of cutting type and potting media on percentage of rooted shoots

There was a significant (p>0.05) interaction between stem cutting type and media on rooting of *Acalypha hispida* (Table 2). All the types of stem cuttings planted on 1:1 sand: coir dust showed highest rooting percentage (100%) compared to others. Those were not significantly different with hardwood and shoot tip cuttings planted in pure sand and pure coir dust. Soft wood cutting planted in pure sand showed lowest performance in rooting (Table 2). This results indicated that the growing medium is the most influencing factor on rooting. Sand with coir dust enhanced the rooting ability of all the type of cuttings.These results were in agreement with the findings of Lokesha *et al.* (1988). It is reported that higher percentage of rooting in *Acalypha* was found in coir dust when compared to sand. Studies on Bougainvillea by Singh *et al.* (2020) showed that, the hardwood cuttingsplanted in different growing media were found significantly better than the control with respect to rooting of cuttings.

A study on propagation of *Ricinodendron-heudelotti* Baill cuttings belongs to family Euphorbiacae by Tchinda *et al.* (2013) showed that, cutting type had significant effects on individual rooting of the accession and it is responsible for almost 70% of obtained results for rooting rate, leaf number and root length. Further, they mentioned that, rooting is generally depending on potting media, cutting type, pre-treatment and harvesting time.

Effect of cutting type and potting media on root weight

As shown in Table 2, significant (p>0.05) interaction was found between the cutting type and media on root weight. There was no significant root weights were found where hardwood cuttings planted in sand: coir dust (0.16g) and pure coir dust (0.15g). Lowest performances were found in all the types of cuttings planted in pure sand.The hardwood cuttings showed better performance in root weight possibly due to its effectiveness and availability of reserved materials for growth of root.

As indicated by Ramtin *et al.* (2011), a study on *Poinsettia pulcherrima* L. proved that the type of cutting had a great effect on the rooting of Poinsettia and lower cutting (hardwood cutting) was identified as the most suitable for rooting. Higher food reserves in the hardwood cuttings enhanced the root development.The results obtained in this experiment may be due to coir dust

and sand when combine together itpromotes faster root growth and gave quicker anchorage to young roots. This mixture may help in retaining air, plant food and moisture and releasing them as when the plant requires**.** Influence of medium is felt before rooting occurs due to water retention and aeration properties which ultimately increase percentage, length and quality of roots (Fagge and Manga, 2011).

Effect of cutting type and potting media on number of leaves

The stem cutting and media showed significant (P>0.05) interaction on average number of leaves produced (Table 2). Hardwood cutting planted in 1: 1 ratio of sand, coir dust and pure coir dust have produced highest average number of leaves (13.43, 13.10). Lowest number of leaves (1) obtained in shoot tip cuttings planted in pure coir dust.An experiment on *Zinnia elegans* by Riaz *et al.* (2008) mentioned that, in general, mixture of silt + leaf manure + coconut compost (1:1:1) gave the highest values of growth parameter such as number of leaves per plant. Further indicated by Dewayne *et al*. (2003) both physical and chemical characteristics of the growth medium exert substantial effect on growth of plants. Physical characteristics of aeration and water holding capacity are probably the most important factors while, the chemical characteristics such as nutritional status, and salinity level have a crucial role on plant development (Dewayne *et al.,* 2003).

Table 2: Interaction effects of cutting type and media on percentage of shoot produced roots, number of leaves, shoot length and root weight

Media types (M)	Cutting types (C)	% of shoot produced roots	Root weight (g)	No. of leaves	Shoot length (cm)
Sand	Tip	100^{a}	0.03^{ef}	2^{de}	0.36^{ef}
	Softwood	0^{d}	0^{f}	2^{de}	0.08^{f}
	Semihardwood	16.67^{c}	0.05^{f}	2.75^{de}	0.43^{ef}
	Hardwood	100^{a}	0.01^{f}	8.75^{b}	1.20^{de}
Coir dust	Tip	100^{a}	0.02^{ef}	1^{e}	0.30^{f}
	Softwood	75^{ab}	0.08^{bcd}	5.48^{c}	1.20^{de}
	Semihardwood	50^{bc}	0.05^{de}	5.65^{c}	1.54^{cd}
	Hardwood	100^{a}	0.15^{a}	13.10^{a}	3.53^{a}
Sand + Coir dust	Tip	100^{a}	0.09^{bc}	3^{d}	0.16^{f}
	Softwood	100^{a}	0.05^{cde}	5.38^{c}	2.16^{bc}
	Semihardwood	100^{a}	0.12^{b}	8.30^{b}	2.40^{b}
	Hardwood	100^{a}	0.16^{a}	13.43^{a}	3.34^{a}
Significant	M × C	*	*	*	*
	M	*	*	*	*
	C	*	*	*	*

Means followed by the different superscripts in a same column are significantly different at DMRT 5%. * Represents significant at 5% and ns represents not significant.

Effect of cutting type and potting media on shoot length

There was a significant (p>0.05) interaction between the factors tested on length of the shoot. Hardwood cuttings planted in pure coir dust and 1:1 sand: coir dust obtained the longest shoot length (Table 2) with 3.53cm, 3.34 cm, respectively. Lowest shoot length (0.08cm) was found where softwood cutting planted in pure sand.The hardwood cuttings of *Acalypha hispida* showed better growth, possibly due to higher reserves in the tissues. Hardwood part of the stem consists of materials necessary for stem growth including nucleic acids, proteins and natural hormones among them, Indole acetic acid which exists in all kinds of plants. It was reported that coir dust possesses good water holding capacity and releases phenolic compounds which promote shoot initiation and better performances of cuttings (Lokesha *et al.,* 1988; Smith, 1955).

Coir dust mixed with potting media helps in maintaining the appropriate texture of the growing media and prevents compaction, thereby resulting in better root growth and shoot growth (Fagge and Manga, 2011). A study on *Antidesma bunius,* belongs to family Euphorbiacae, by Totaan (2019) is on par with the results of *Acalypha hispida*. The study showed that basal section of the stem cuttings (hardwood type of cutting) obtained highest shoot length followed by middle section (semi hardwood). The basal section of *Antidesma bunius* stem cuttings has thicker and larger diameter as compared to the apical and middle portion that could probably produce the longer shoot length.

CONCLUSIONS

The present study showed that there was significant variability in shoot and root development of the cutting type with media. Hardwood cuttings planted in the media contained sand mixed with coir dust at the ratio of 1:1 and pure coir dust media showed the significantly highest performances in most tested parameters. Therefore, *Acalypha hispida* plants steadily be propagated with highest survival rate and rooting percentage using hardwood cuttings planted in sand: coir dust 1:1 mixture or pure coir dust media.

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Observations on relationship of seed content with russeting and fruit physical characteristics of apple cv. Golden Delicious

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ABSTRACT

Apple (Malus x domestica Borkh.) seeds during their development in the fruit, produce a sequence of different types of hormones,influencing fruit growth and quality in diverse ways.This way role of seeds can also be postulated in fruit russeting of apple. So this study was framed to identify the relationship of fruit seed content with qualitative factors like length, diameter and weight,and also russeting incidence in apple cv. 'Golden Delicious'. Though seed content of the fruit seemed to influence significantly all the above factors, but could only explain the variability in russeting up to 29%, which indicates that there may be many more factors associated with fruit russeting of 'Golden Delicious' apple. The study indicated that weight, length and fruit diameter were relatively strongly related to the seed content explaining the variability of 43%, 45% and 46% respectively. Moreover, russeting also influenced negatively the fruit size factors in association with seed number.

Keywords: Fruit quality, fruit size, pollination, russeting, seed number

INTRODUCTION

Pollination is one of the first and most important steps in fruit production. Often low yield and/or poor fruit quality are attributed to poor pollination (Garratt *et al*. 2014). Pollination as such ultimately leads to fertilization and seed development, and influences the number and distribution of seeds within the fruit, which has long been known to influence fruit quality and quantity (Drazeta, 2002, Garratt *et al*. 2014). At least ten pollen grains are needed to produce a full complement of seeds for most apple cultivars, though due to varying levels of non-viable pollen and genetic incompatibility issues inherent with orchards, many more grains are usually required for seed set (Sheffield *et al*., 2005). It is accepted that seeds play a role in sink strength probably through the production of hormones (Balaguera-Lopez *et al*.,2020). Therefore, one can expect that sink strength (and thus fruit physical characteristics) of apple fruits depends partially on seed number.

A crop of small apples is worth less than the same weight of larger apples, thus the individual fruit size needs to be taken into account in accessing crop performance. In multi-seeded fruits, weight sometimes can be related to the number of seeds which develop. There is an upper limit to seed number, set by the number of ovules formed during flower development, and in some fruits a limit to tissue development associated with each seed can be postulated. These factors may determine the maximum weight which fruit can attain, against which actual weights can be compared. Any shortage would then be accounted for by poor pollination or tissue development, or a combination of both (Rehman *et al*., 2018).

Apple seeds during their development in the fruit, produce a sequence of different types of hormones, the appearance of which is linked with successive stages in the development of the endosperm and embryo. The development of a free nuclear endosperm is characterized as first stage, and is associated with the presence of cytokinins (Zhang *et al.,* 2020).Then after 4 to 5 weeks, the first stage is terminated with the development of cellular primary endosperm, where in auxins can be noticed. Several GA's have also been confirmed

to be present during this second stage, peaking at the time when embryo has almost reached its final size (Bermejo *et al*., 2018).

In view of the hormone-directed transport, it seems reasonable to suggest that one of the main functions of the relatively high concentrations of hormones found in developing seeds may be the mobilization of essential metabolites – particularly carbohydrates and soluble nitrogen – against the competing demands of the growing shoots. It is certainly true that fruits with no seeds or with only a low seed content are not normally able to survive this competition, though they can be made to develop if competing growth is suppressed (Abbott, 1960).

There is also strong circumstantial evidence that gibberellins translocated from the seeds to the bourse may inhibit flower initiation in the bourse buds, thus giving rise to a phenomenon where it can also be translocated to the epidermis and help in controlling the russet formation. Hence, this study was aimed to find out the role of fruit seed content in defining the fruit physical characteristics and russeting disorder of Golden Delicious apple fruit.

MATERIALS AND METHODS

The present study was carried out at a private orchard in Shalimar (34º08'54" towards North and 74º53'03" towards East), near Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Srinagar, Jammu and Kashmir during the year 2018. Kashmir represents the temperate climatic conditions. The maximum and minimum temperature of valley during the growing season ranged from 23ºC and 29.9ºC and -5.8ºC to 12ºC, respectively with relative humidity of 43.9% and 650-800 mm rainfall mostly received during the period December to April. Trees used in this experiment were 18 year old "Golden Delicious" on seedling rootstock. The trees were spaced at 6m x 6m on clay loamy soil. All the Trees were managed according to the normal and uniform management practices. Russeted and russet free fruits (104 in number) were randomly selected covering all the four directions at harvest. Fruit length and diameter were measured using digital vernier caliper, and the weight using digital balance. Approximate Russet percentage of each fruit was calculated using the formulae:

$$Russet\% = \frac{Russeted\ area}{Total\ fruit\ surface\ area} x100.$$

Fruits were cut into two halves and the seed count was noted for each fruit.

Regression analyses were conducted using the 'lm' procedure of the R statistical software. The regressor (seed number) was evaluated for its effects on the russeting percentage, fruit length, diameter and weight. Moreover, combined effects of russeting and seed number were also tested in relation to fruit length, diameter and weight.

RESULTS AND DISCUSSION

Fruit characteristics of apple cv. Golden Delicious, in this study were found to be influenced by the seed number. All the four response variables (russeting, fruit length, fruit diameter and fruit weight) were significantly ($P<0.001$) related to fruit seed content. Fruit length, fruit diameter and fruit weight showed a positive correlation with the seed count. Whereas, the russeting percentage was negatively correlated with seed number *i.e.* with the increase in fruit seed number, the russeting percentage was reduced (Fig. 1). However, fruit russet percentage showed a poor ($R^2<0.29$) relationship with seed content (SN) (Fig.2). Golden delicious apple are susceptible to russeting. It has been observed that application of exogenous gibberellin's, controls russeting in apple to a great extent(Taylor *et al.,* 1986; Pesteanu, 2018), but at the same time immature apple seeds are exceptionally rich sources of gibberellins: $GA_{1,}$ $GA_{3,}$ $GA_{4,}$ $GA_{7,}$ GA_{20} andGA_{34}(Stephen *et al*., 1999). Seed number on fruit russeting could only explain variability of 29% in this case. Thus it is hypothesized that seeds affect the sink of individual fruits, probably through hormones in a quantitative and qualitative way. Here fruit epidermal features specific to the particular variety must be influencing the relation. Moreover, environment, location and other unidentified factors may account for the unexplained variability of the results.

The relationships of seed number with the variables, fruit length, diameter and weight were little stronger ($R^2< 0.45$), ($R^2< 0.46$) and ($R^2< 0.43$) respectively (Fig.3 – 5). Further, fruit weight was

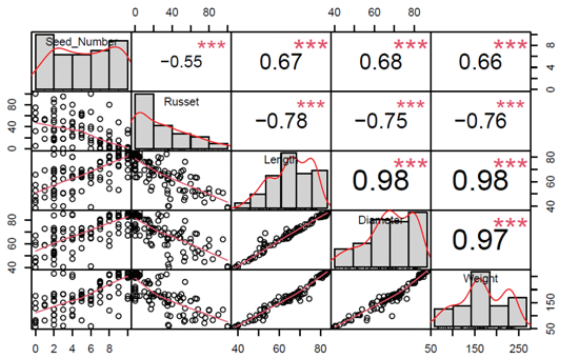

Fig. 1: Correlation plot of seed number, russeting, fruit length, diameter and weight of Golden Delicious apple.

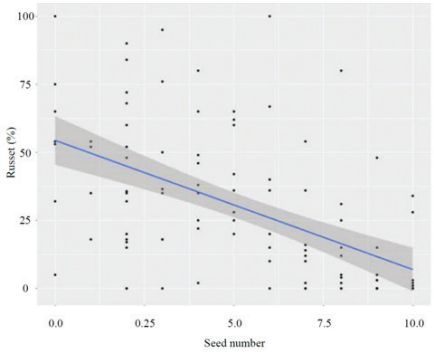

Fig. 2: Relationship between Russet % and seed content of Apple cv. Golden Delicious, Regression line Y = 54.4-4.746*X, r^2=0.29, n=104

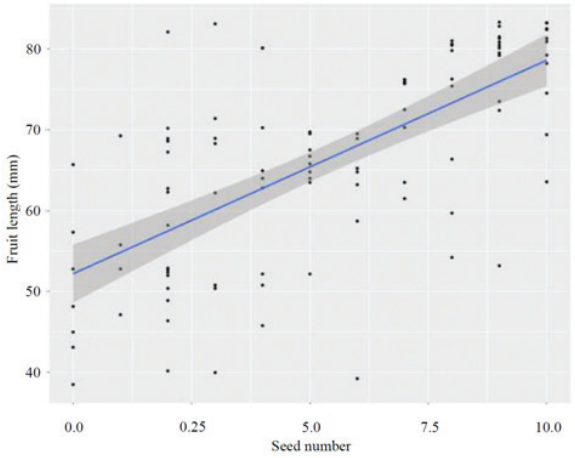

Fig. 3: Relationship between fruit length and seed content of Apple cv. Golden Delicious, Regression line Y = 52.21+2.639*X, r^2=0.45, n=104

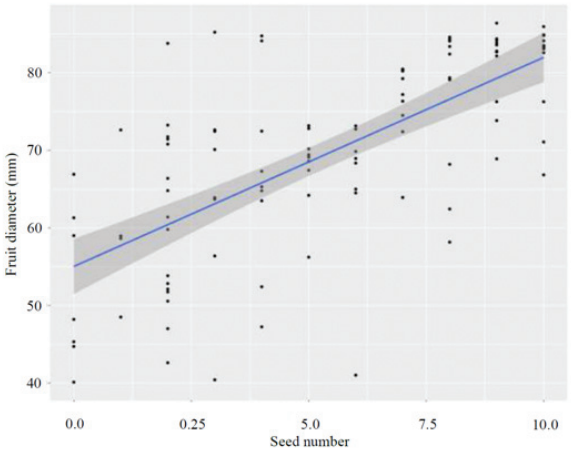

Fig. 4: Relationship between fruit diameter and seed content of Apple cv. Golden Delicious, Regression line Y = 55.02+2.697*X, r^2=0.46, n=104

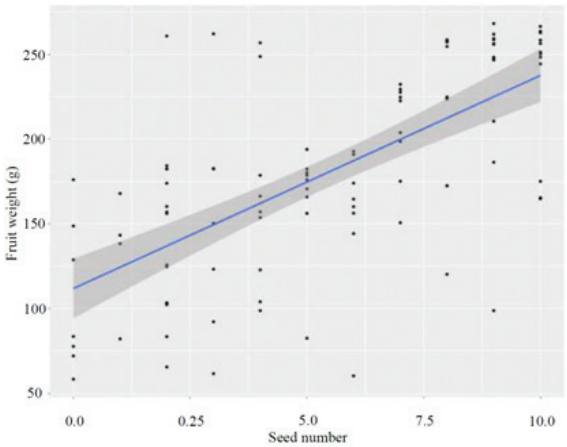

Fig. 5: Relationship between fruit weight and seed content of Apple cv. Golden Delicious, Regression line Y = 111.79+12.58*X, r^2=0.43, n=104

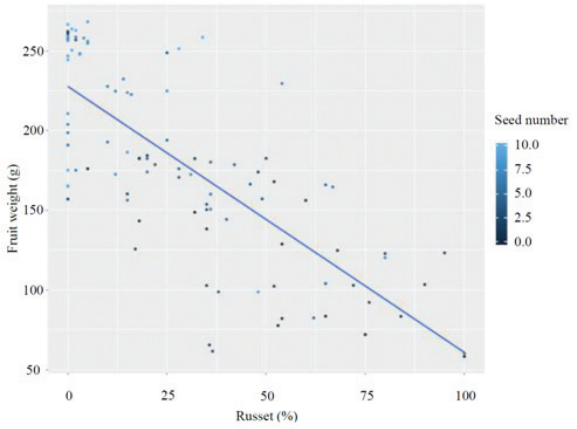

Fig.6: Regression plot of fruit weight on two predictor variables (russet and seed number) of Apple cv. Golden Delicious, Regression line Y = 180.26+6.58*(seed number) 1.263* X+ 0.002* X* (seed number), r^2=0.66, n=104

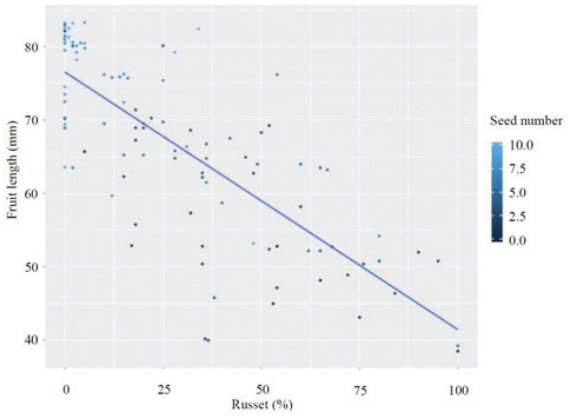

Fig.7: Regression plot of fruit length on two predictor variables (russet and seed number) of Apple cv. Golden Delicious, Regression line Y = 67.34+1.26*(seed number)-0.29*X+0.005* X*(seed number), r^2=0.68, n=104

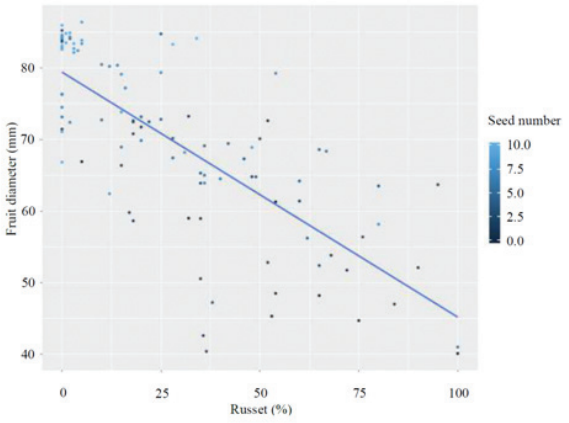

Fig. 8: Regression plot of fruit diameter on two predictor variables (russet and seed number) of Apple cv. Golden Delicious, Regression line Y = 70.05+1.27*(seed number)-0.3* X+0.011* X*(seed number), r^2=0.67, n=104

more strongly (R^2<0.66) related when the combination of russet % and seed content were taken into consideration. Same sort of relationships for fruit length (R^2< 0.68) and diameter (R^2< 0.67) were observed with the combination of russet % and seed content.

Seed content could explain the variability of fruit length and diameter up to 45% and 46% respectively. Seed number explained 43% of variability in fruit weight of the cultivar'Golden Delicious'. The increase in fruit weight with increasing plump seed number agrees with reports of Ward *et al.*(2001). The results described here suggest that the sink strength of apple crop cv. 'Golden Delicious' changes in nearly, but not precisely, directly proportional to the number of apple seeds.Seed distribution resulting from pollination levels, dictates fruit quality. The growth of a particular sector is principally influenced by the seeds within its locule. Each carpel exerts a somewhat autonomous effect on fruit growth and, at least partially, develops as a unit. There is a week seed influence with increasing distance from the sector indicating that seeds principally govern fruit development (Drazeta *et al*.,2004). Scheffield (2014) clearly demonstrated and suggested that fruit growth in response to seed presence occurs primarily on a single plane – outward horizontally from the carpels and contributes to increased fruit diameter.

The explanations were more strong (68% for length and 67% for diameter) when seed number was taken in combination with russeting percentage. Same was the case with fruit weight, where again the variability was more strongly (66%) explained with the combination of factors, russet% and seed number. This clearly indicates that russeting too may be influencing inversely the fruit size factors of 'Golden Delicious' apple fruit.

Conclusion

The study leads us to conclude that fruit size features like length, diameter and weight are influenced by the seed content of that fruit in a positive way. The russeting disorder in 'Golden Delicious' apple can be reduced to some degree with the enhancement of fruit seed number, by means of proper pollination. Though, there may be many more factors contributing towards structuring all these parameters. Further, russeting also has a role in reducing the size features of the fruit.

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SHORT COMMUNICATION

Momordica dioica Roxb (Spine Gourd)- An underutilized vegetable and medicinal plant in Sri Lanka

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ABSTRACT

Momordica dioica Roxb belongs to the family Cucurbitaceaeis a wild, perennial and creeping plant growing in the tropical and subtropical countries; Pakistan, China, India, Nepal and Sri Lanka. It is well known as folk medicine and nutritious vegetable as it contains various nutrients and phyto-chemicals with huge medicinal value. Different plant parts of M. dioica provide a number of phyto-constituents; alkaloids, steroids, triterpenoids, flavonoids, glycosides, ursolic acid, vitamins, minerals and rich in fibre. They are potential to cure asthma, excessive salivation and inflammation caused by lizards, snake bite, elephantiasis, fever, mental and digestive disorders and maintain skin health. According to the indigenous knowledge spine gourd plays a vital role in reducing various disorders like urinary disorders, digestive disease and as a remedy for bleeding. At the present scenario, M. dioica is considered as an underutilized, non-neglected plant in Sri Lanka. Extensive identification of M. dioica and its phyto-constituents provide huge potential to protect the plant biodiversity and enhance medicinal utilization. The paper highlighted the nutrient content, different uses, botany and cultivation aspects of M. dioica which will be helpful for the students and the interested peoples.

***Keywords*:** Green medicine, *Momordica dioica,* phyto-constituents, spine gourd, underutilized vegetables.

INTRODUCTION

Use of synthetic drugs create the global health hazard and enhances the possibility of cancer, diabetes, neurodegenerative disorders. As a remedy for that, there is an urgent need for production of medicines using the natural herbs. Indigenous medicinal provide the favourable solution for the global healthhazard by reducing the negative impact of the synthetic drugs (Jha *et al.,* 2017). *Momordica dioica* is a plant that considered as folk medicinal and nutrient rich vegetable. It contains a number specific constitution, called as phyto-constituents (alkaloids, tannins, fixed oil, flavonoids, sterol and amino acids) (Anjana *et al*., 2020). In Sri Lanka,it is a kind of underutilized vegetable but, according to the Thiruvengadam *et al*. (2011) there is a high demand in East India. It is considered a higher protein containing fruit in the *Cucurbitaceous* family (Bharathi *et al.*, 2010). Spine gourds are very famous because of native bitter taste, due to the presence of phytochemicals like Triterpenes Momordicin, Lectins, Â-Sitosterol, Saponin, Glycosides are few of these alkaloid compound cause for its native bitterness (Jha *et al.,* 2017).

Many researchers pay attention to developed new *Momordica dioica* varieties by up gradating the existing poor qualities. Indira Kantola I (RMF 37) is a new commercial variety developed by the Indira Gandhi Agricultural University in India. It was resistant to the major pest and can have harvest within 35- 40 days after the cultivation (Anjana *et al.,* 2020). In the present situation underutilized useful plants like *Momordica dioica* face the extinction challenge (Dahanayake, 2015). Therefore; there should be proper conservation measures and awareness programme about nutritional and medicinal value of these plants.

BOTANICAL DESCRIPTION

Momordica dioica is a kind of vine, flowers are born during June to July and fruiting period from November to September. Male and female flowers are borne separately, so called as monosexual (Hitinayake *et al*., 2017). According to the literatures, around 22% of possibility to fruit set under the normal environment conditions and 100% possibility under the hand pollination conditions (Sandilya *et al.,* 2019). Every node produces male and female floral buds. Male buds are produced during the second week of the August and continue to the first week of October. Female flowers are

Fig. 1:Morphology of *M. dioica* leaf (simple leaf with deep lobes)

Fig. 2: Morphology of *M. dioica* fruits (short beak with soft spines)

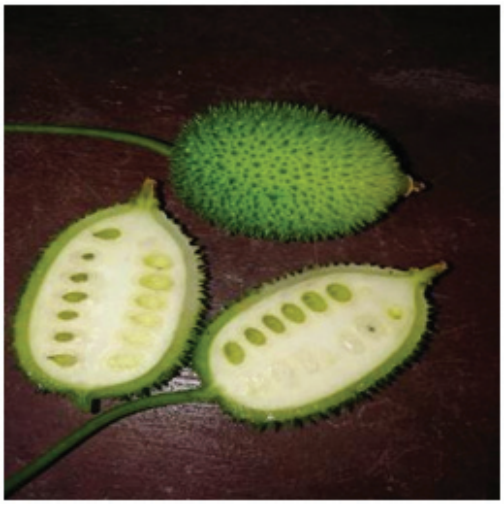

Fig. 3: Longitudinal section of *M. dioica* (axil placentation)

Fig. 4: Cross section of *M. dioica.*

Fig. 5: Morphology of male flower

Fig. 6: Morphology of the female flower

open during the first - second week of September to third week of October (Sandilya *el al.,* 2019). Male flowers are light yellow in colour, long around 2.8 cm. Petals with the shape of oblong or lanceolate and consist of five calyx with linear or lanceolate with five corolla and three stamen. Female flowers consist of small yellow colour bracts having three nectar glands (Bawara *et al.,* 2010). According to Sandilya *el al.*(2019), male flowers are open around 4.00 am and female flowers around 6.00 am. Therefore 5.00-6.00 am is considered as best period for the hand pollination. Fruit sizes are 2-3 cm in diameter and 2.9 to 5 g weight. Fruits are short beaked and exocarp is soft, present the spines (Jha *et al.,* 2017). Pods are green during immature and turn to yellow, light green during maturity (Salvi and Katewa, 2015). Ovules are arranged along the free central column of the fruit and seeds are covered with the regulated and hard endocarp (Tissa *et al.,* 2013) because of that, it shows tolerance against the caterpillars;pumping caterpillar, gall fly and root knot nematodes(Anant *et al.,* 2019).

Leaves are simple and broadly ovate with deep lobes in outline, generally length is in between 3.8 to 10 cm (Bawara *et al.,* 2010). Stem round and branched, furrowed one and elongated tendrils are present (Sandilya *et al.,* 2019).

Utilization of *Momordica dioica*

Several studies have been confirmed that *M.dioica* is a nutritious vegetable and used as folk medicine (Salvi and Katewa**,** 2015; Nawarathna *et al.,* 2020). According to Bharathi *et al.* (2010), kernels of the seeds are used for the dying oil for varnish industry. Hexane, an extracted compound, showed the anti-feeding effect of *Spodoptera litura*. Seed oil of the *Momordica* shows the insecticidal effect 100% mortality with the 4% seed oil concentration. *M. dioica* contains a number of phyto-constituents, bioactive compounds with numerous propertiesare important in the western medicinal treatments and used as insecticidal effects too (Table 1).

Table 1: Phyto-constituents of different plant parts of *M. dioica* and their effect

Plant parts	Extracted compound	Effect of each compound
Fruits	Hexane extract	Anti-inflammatory property, Neuro-protective ability
	Methanol extract	Protect the liver cells from damages on hepatocytes , anticancer effect
	n-butanol extract	Reduction of pancreatic lipase activities
	Hexane and ethyl acetate extract	Provide protection against the anti-feeding activity of cotton leaf worm, Anti-diabetic and antidepressant properties
Leaves	Methanol extract	Hepato-protective,Anti-hepatotoxicity effect
	Aqueous extract	Allelopathic activity on seedling growth, seed germination
Root	Alcoholic extraction	Inhibit the formation of free oxygen radical
	Ethanol extraction	Inhibit the growth, Anti-fertility effect
	Methanol	Anticancer effect
Seeds	Seed oil	Provide protectant against *Callosobruchus chinensis*, Antiallergic effect

(Source: Talukdar *et al.,* 2014; Jha *et al.,* 2017).

Nutritional values

According to the available reports, three triterpenes and two steroids compounds could be isolated from the *M. dioica* fruit (Jha *et al.,* 2017; Talukdar *et al*., 2014). Phytochemical studies indicated that spine gourd have high nutritional value which contain proteins, triterpenes and high amount of vitamin C, iodine, alkaloid, flavonoids, glycosides, amino acids and trace of manganese (Talukdar *et al*., 2014); carotene, thiamine, riboflavin and niacin (Salvi and Katewa, 2015) and good source of chromium and zinc (Talukdar *et al.,* 2014). Results showed that 50g of edible fruit contain 42.1% moisture, 3.35g carbohydrate, 1.9g protein, 1.56 g fat, 1.5g fibre and 0.5 g minerals calcium 16.5 mg, iron 2.3mg and phosphorous 4.21mg (Jha *et al.,* 2017).

Medicinal value

Antioxidant Activity: *M. dioica* have compounds with antioxidant property and have the ability inhibit the formation of oxygen derived free radicals and protect the cells (Anant *et al.*. 2019).

Fruit extracts have diuretic, alexiteric stomachic, laxative, hepatoprotective, and antivenom properties. It is used to cure asthma, leprosy, excessive salivation (Bawara *et al*., 2010) and to prevent the inflammation caused by lizard, snake bite, fever, mental, digestive disorders and troubles of heart. Because of these properties fruits are used for treating the pimples and acnes on the skin (Talukdar *et al.,* 2014).

Anticancer activity: According to the report of Anjana *et al.*(2020), root extracts have different constitutions with anti-cancer property. Á-spinasterol-3-o-â-D-glucopyranoside is the one of major compound that show effect against the cancer cells (Jha *et al.,* 2017; Talukdar *et al.,* 2014).

Antifertility effect: Talukdar *et al.*(2014), mentioned that *M. diocia* have effect on anti-fertility. He concluded that fruit extracts have ability to induces the anti-fertility effects on female rats while have no effect on male rats.

Allelopathic activity: *M. dioica* seed oil has naturally insecticidal properties. By spraying the extracted oil on the cereal grain, it provides anti-feed activity against the cereals feed insects (Anjana *et al.,* 2020).

Ayurvedic values

Ancient peoples used *M. dioica* as a folk medicine other than the vegetable (Anjana *et al.,* 2020). Spine gourd root juices contain the antidiabetic, anti-inflammatory effect and extract of the spine gourd leaves applying on the head is better treatment for the headache. When applied, root extracts over the whole body provides the superficial effect for high fever. Oral administration of the leaf past is used for many skin diseases as pimples, acnes and softening the skin (Talukdar *et al.,* 2014). *M. diocia* is the most effective nutrient vegetable for the children, lactating and pregnant mothers because, it creates good strength on the immune system in the body (Salvi and Katewa, 2015), used to treatment the eye diseases and as a medicine for diabetes (Jha *et al.,* 2017).

Cultivation

It is a worm seasonal crop, successfully cultivated in the subtropical and tropical regions. Plenty of sunshine and low humidity conditions with well drained sandy loam soil with neutral pH value (between 5.5- 6.5) provide a comfortable zone for the plant growth. Around 27- 33^{0}C is ideal condition for the maximum growth and yield of the plant (Ponnusamy and Balusamy, 2019). Before planting, the land should be well ploughing with organic manure or farm yard manure. The plant spacing will be at 1-2 m between two plants and seeds are sown at a depth of 1-2 cm. Plants are highly susceptible to water logging and also affected by drought condition. Insect and pest damage is correlated with the environment factors. Insect pests population is more during August-September and make considerable damage to the plants (Anant *et al*., 2019). Fruit fly (*Bactrocera cucurbitae*) are the most devastating group of pests that affect on the potential yield of the *Marmodica.* Red pumpkin beetle, leaf eating caterpillar, cucumber moth and hairy caterpillar are some of threatening insects for the *dioica* (Anant *et al.,* 2019).

Propagation

M. dioica can be propagated by seeds, cutting (Nawarathna *et al*., 2020) or tuber (Jha *et al.,* 2017). Seeds have dormancy for 5-6 months. Dipping the seeds in water for a day at the 30^{0}C showed better germination (Thiruvengadam *et al.,* 2011). The cutting of terminal vine with 2-3 nodes is the best portion for easily rooting (Ponnusamy *et al*., 2019). 70-80 days after sowing plants reach commercial maturity (Anjana *et al*., 2020). Tissue culture is another propagation way that was identified recently. There are four types of explants, *M. dioica* node, shoot tip, leaf and the cotyledon; cotyledons are the best propagating material. MS media with the combination of 0.1mg / 1NAA and 1.0mg/1BAP shows the best performances for the callus formation. MSHP +Ads+ IBA +Agar are the best combination treatment combination for root development (Deokar *et al*., 2003). Other than conventional breeding methods, direct organogenesis is the novel method for the propagation of *diocia* (Thiruvengadam and Chung, 2011).

Conclusion

Nearly 90% of global food demand is fulfilled by the few of dominated crops worldwide. Another 10% is covered by the rest of the crop types, which

are considered as the underutilized and non-neglected crops. The majority of these underutilized crops have significant nutritional and the health benefits. Also have a long history as its uses in the Ayurvedic industry. Most of these underutilized crops are dominant in the rural agriculture and provide to the rural household income, food and nutritional security. *M.dioica* is a medicinal and nutritious vegetable with capability to grow in the limited space even as a potted plant with simple potting media.

M. dioica is a kind of wild relative of *Momordica* plant spp, there are a number of identified poor qualities such as small size fruit, low yield, flowers ratio are not synchronized properly resulting yield of low quality and quantity. Breeders should pay their attention to develop the new *dioica spp*, by replacing the unwanted wild features. As the fruits are seasonal and limited availability, there is huge potential to prepare the value added and nutritious products.

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SHORT COMMUNICATION

Improvement of grafting success of mango through application of plant growth regulators

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ABSTRACT

The mango is generally propagated by grafting and grafting success depends on several factors. Physiological condition of scion and rootstock plays a vital role in grafting union where external application of plant growth regulators may be helpful to gear up better cambium activity for making grafting joint. Considering beneficial role of plant growth regulators, an investigation was carried out in a nursery of private farm during the year 2015 with different concentrations of NAA (10 ppm and 20 ppm) and GA_3 (5 ppm and 10 ppm) were applied through spray applications at different time intervals (1 day after grafting followed by 21 days after grafting and 7 days after grafting followed by 21 days after grafting) on grafts using scion of mango variety Mallika. The experiment was laid out in factorial Randomized Block Design (RBD) with five replications. The result showed that GA_3 @10 ppm resulted in highest grafting success (56.05%) with better growth of sprouted shoots. However, leaf number of sprouted shoots was comparatively lower in GA_3 treatment irrespective of doses. In case of all traits under study time of growth regulator application was significantly important irrespective of growth regulators and application of growth regulators at 1 day after grafting followed by the second spray at 21 days after grafting gave the better result. Therefore, GA_3 @10 ppm applied at 1 day and 21 days after grafting is recommended to improve grafting success in Mallika cultivar of mango.

Keywords: Mango grafting, GA_3, NAA, time of application

INTRODUCTION

Mango (*Mangifera indica* L.) 'The king of fruits' and is the choicest fruit crop of India for its rich taste, flavor, color, large scale production and diverse end usage. Mangoes contain antioxidants such as quercetin, fisetin, isoquercitrin, astragalin, gallic acid and methyl gallate. All these properties protect our body against breast cancer, colon cancer, prostate cancer and leukaemia. It contains high level of vitamin C, fibre and pectin making it a perfect fruit that helps in controlling high cholesterol level. It also contains vitamin C, A and other different kinds of carotenoids. All these essential nutrients are beneficial for our immune system keeping it strong and healthy. Mallika is one of the important regular bearer hybrid varieties that got research attention among scientists and is popular among growers.

Mango being a highly heterozygous fruit crop is preferred to propagate through vegetative methods and among different types of vegetative propagation techniques grafting is the most common one and it resulted good success as compared to other methods (Ghosh and Bera, 2015). Successful grafting requires tissue reunion including formation of vascular connections between the stock and scion. Grafting initially triggers the secretion of pectin from cells at the cut site to adhere the rootstock and scion together. Dedifferentiated stem-cell like tissue, termed callus, then forms at both junctions until the grafted tissues join and plasmodesmata can bridge the connection site. Cambium, cortex and pith cells surrounding the phloem and xylem divide and, together with callus cells, differentiate into vascular tissues and connect the two junctions (Nanda and Melnyk, 2018). Effect of growth regulators as potential candidates involved in grafting process has been well acknowledged. Of these the most important hormones are auxin and cytokinin that play an important role in vascular system formation during root development and differentiation of vasculature in callus. Cytokinin accumulates in the rootstock and auxins accumulate in the scion portion due to the cutting process that consequently activates the genes associates with wound response and vascular development (Melnyk and Meyerowitz, 2015). On the other hand gibberellin like substances has a

significant role in plant growth regulation (Brian, 1959). Use of plant growth regulators in cuttings and layering in fruit crop propagation has been established but information regarding use of plant growth regulators in improving success in grafting or budding in different crops is scanty. It is well known that grafting success between compatible scion and rootstock type is depend upon the professional skill of grafting, external environment and management practices followed after grafting. Considering beneficial role of auxin and gibberellins in regulating cell physiology particularly in meristematic activities, the present experiment was conducted to find out any key role of these hormones in improving grafting success in mango.

MATERIALS AND METHODS

The present investigation was carried out in a nursery of a private farm at Jhargram of Paschim Medenipur of West Bengal during the year 2015. Climatic condition of area of study was dry subtropical. Different concentration of growth regulators and different application time were the two experimental factors in this research study. The first factor represented five different concentrations of growth regulators (T_1- NAA @10 ppm, T_2- NAA @20 ppm, T_3- @$GA_3$5 ppm, T_4- @GA_3-10 ppm, T_5- Control or water spray). The second factor was two different application time of growth regulators (S_1- 7 days after grafting followed by the second spray at 21 days after grafting and S_2- 1 day after grafting followed by the second spray at 21 days after grafting). 14- months old healthy seedlings of local mango, grown in polybags were used as rootstock. Soft wood grafting technique was followed taking scions of Mallika mango cultivar. Five graft samples were taken in each replication. Grafting was done in the first week of September. All the grafted plants were kept under semi-shade condition after grafting for one month then shifted to open condition. Observations were recorded for the three parameters; *viz*., percentage of grafting success, height of sprouted shoots and leaf number, noted three months after grafting. The percentage of graft success estimated at 3 months after grafting and computed by the following formula:

$$\text{Percentage of graft success (\%)} = \frac{\text{Number of success graft}}{\text{Total number of graft done}} \times 100$$

The data were statistically analyzed by factorial randomized block design (RBD) with five replicates, and each replicate included 5 samples per treatment. The means for all the treatments were calculated; analysis of variances (ANOVA) was performed by using SPSS version 24.0 at $P < 0.05$ probability level is regarded as statistically significant. Duncan Multiple Range Test (DMRT) (Duncan, 1955) was conducted to compare effect of different plant growth regulator combinations and date of spray on grafting success rate, height of sprouted shoots and leaf number of sprouted shoots.

RESULTS AND DISCUSSION

Grafting success

The percentage of grafting success without any growth regulator treatment was 44.49% which was *at par* with the results of NAA @10 ppm with 44.04%. GA_3 @ 5 ppm treatment resulted in the lowest rate (42.05%) of grafting success among all other treatments while GA @ 10 ppm contributed the maximum rate of grafting success with 56.05%, followed by NAA @20 ppm with 48.09% grafting success rate (Table 1). Time of growth regulator applications also revealed significant difference. The growth regulator application at 1day after grafting followed by the second spray at 21 days after grafting showed 48.36% grafting success while the growth regulator application at 7days after grafting followed by the second spray at 21 days after grafting showed 46.75% grafting success and the lowest grafting success of 44.49 % was noted in control. The different doses of growth regulator application and at different time interval were highly significant in case of GA @10 ppm while dose and time of growth regulator application showed no significant differences in case of GA @ 5 ppm as presented in Figure (1. a). Role of plant growth regulators in improving grafting success may be explained from the fact that phyto-hormones act as signal molecules at the sites of action resulting into cell growth and tissue differentiation, especially at the graft interface (Aloni *et al.,* 2010). Thus influence the scion–rootstock relationship by signalling both above and below graft union (Kondo *et al*., 2014). Growth regulator like auxin released from the vascular bundle of stock and scion induces compatible unions through differentiation of vascular tissues, act as morphogenic substances as a result accelerate

Table 1: Effect of different doses and schedule of application of growth regulators on grafting success, height and leaf number of sprouted shoots

Treatments	Grafting success rate (%)	Height of sprouted shoot (cm)	Leaf number of sprouted shoot
T1 (NAA @10 ppm)	44.04^{bc}	8.15^{a}	8.31^{a}
T2 (NAA @ 20 ppm)	48.09^{b}	7.66^{a}	8.51^{a}
T3 (GA_3 @ 5 ppm)	42.05^{c}	7.09^{b}	7.70^{b}
T4 (GA_3 @ 10 ppm)	56.05^{a}	7.81^{a}	7.51^{b}
T5 (Control)	44.49^{bc}	6.75^{b}	8.34^{a}
S_1	46.75^{b}	6.68^{b}	8.01^{a}
S_2	48.36^{a}	8.68^{a}	8.01^{a}
S_0(Control)	44.49^{b}	6.75^{b}	8.34^{a}

grafting success (Pina and Errea, 2005). On the other hand, gibberellin like substances has a significant role like auxin in natural plant growth regulation (Brian, 1959). Nanda and Melnyk (2018) explained that Gibberellins (GAs) are diterpenephyto hormones with an important role in plant development, particularly in regulating plant growth, as GAs promote cell expansion, cell differentiation and cell proliferation. GAs also stimulate xylogenesis in cambium tissue. The role of GAs in wounding is becoming clearer and appears that GAs are important for cell expansion

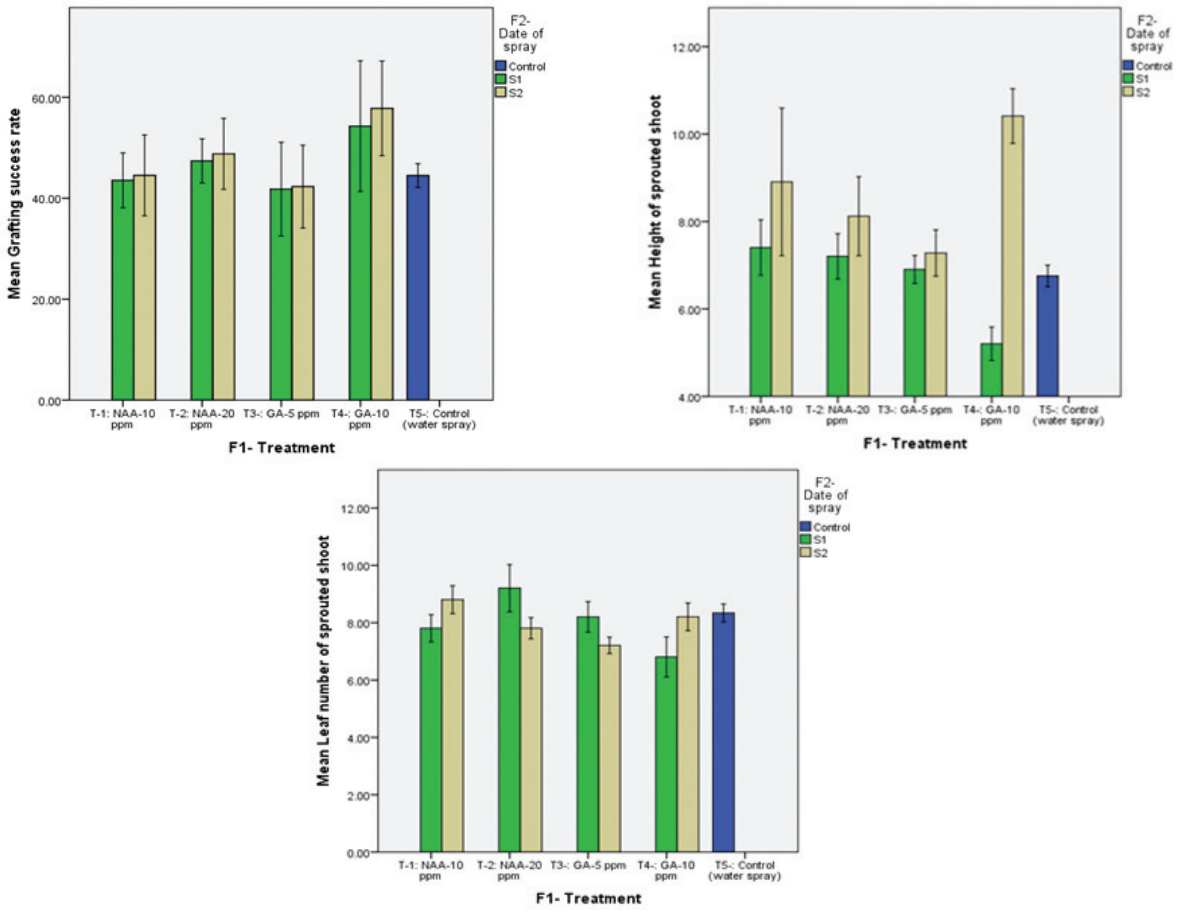

Fig. 1: (a) Effect of different doses and time of growth regulators application on grafting success rate (b) Effect of different doses and time of growth regulators application on height of sprouted shoots (c)Effect of different doses and time of growth regulators application on leaf number of sprouted shoots of Mango cv. Mallika

to seal the wound, whereas auxin is important for vascular tissue proliferation and reconnection across the graft junction. In the present experiment also, application of GA specifically at 10 ppm concentration revealed outstanding result when applied immediately after accomplishment of grafting.

Height of sprouted shoots

Among different growth regulator doses, maximum sprouted shoot height was observed in NAA @ 10 ppm (8.15 cm) which was at par with GA_3 @ 10 ppm (7.81 cm) and NAA @ 20 ppm (7.66 cm) and lowest in control (6.75 cm) (Table 1). Different time of growth regulator application expressed significant differences with height of sprouted shoots.The growth regulator application at 1 day after grafting followed by the second spray at 21 days after grafting showed highest height of sprouted shoot i.e. 8.68 cm while the growth regulator application at 7 days after grafting followed by the second spray at 21 days after grafting showed 6.68 cm in height of sprouted shoot and was at par with no regulator application or control (6.75 cm). Interactions between growth regulators and time of spray in sprouted shoot height was highly significant and maximum shoot height was observed with the application of GA_3 @ 10 ppm when applied at 1 day after grafting followed by the second spray at 21 days after grafting (Fig. 1.b). Different doses of NAA application also revealed significant difference with different time of application and in case of all treatments, the application of growth regulator revealed more effective when applied at 1 day after grafting followed by the second spray at 21 days after grafting(Fig. 1.b). Better shoot growth due to NAA may be due to the fact that auxin influence the production and function of cytokinin, which is produced in roots and translocate to the scion, where it maintains significant plant processes such as shoot growth (Elfving and Visser, 2006).

Leaf number of sprouted shoots

The leaf numbers of sprouted shoots were not influenced by different doses of growth regulators application. However, maximum numbers of leaves in sprouted shoots (8.51) were observed in NAA @ 20 ppm application which was at par with no growth regulator or water applications (8.34) and NAA @ 10 ppm (8.31) respectively. The lowest leaf number of sprouted shoots (7.51) was observed in GA_3 @ 10 ppm which was *at par* (7.70) with GA_3 @ 5 ppm. Similarly, no significant difference was observed among different time of growth regulator applications with control.

CONCLUSION

From the present study, it was revealed that plant bio-regulators have a significant role in improving grafting success and increasing shoot growth. But time of application is important factor to get the desired result. In Mallika cultivar of mango, it was observed that spraying of GA_3 @ 10 ppm immediately after grafting followed by 21 days after grafting significantly influences grafting success and shoot growth.

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