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Antioxidant and antibacterial activity of root extracts of Licorice (*Glycyrrhiza glabra*)

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ABSTRACT

This study has been initiated to reduce burden of Extended-spectrum beta-lactamase (ESBL)-producing Gram negative pathogens using Licorice (Glycyrrhiza glabra) root extracts as natural source of therapeutics molecules. In present investigation, phytochemical analysis of aqueous and methanol extract of Glycyrrhiza glabra was compared as an antioxidant and as antibacterial against ESBL gene containing Escherichia coli. Ascorbic acid for antioxidant activity and cefotaxime for antibacterial potential was taken as standard. The 1,1-Diphenyl-2-picrylhydrazyl (DPPH), and ferric reducing antioxidant power (FRAP) assays indicated that aqueous and methanol extracts have antioxidant activity comparable to ascorbic acid. Methanol extract shows better antibacterial activity than aqueous extract against E. coli. Both extracts do not have significant antibacterial activity when used alone but can be able to enhance efficacy of cefotaxime on synergistic application. Its high antioxidant potential may help immunity to eradicate these infections.

Keywords **:** ESBL; *glabra;* antioxidant; phytochemical; antibacterial; root extract

INTRODUCTION

Gram negative pathogens which can produce Extended-spectrum beta-lactamase (ESBL) have been developed into serious distress over the last few decades. It is considered that ESBLs will definitely be able to create significant therapeutic problems in the future. Literature showed that Extended-spectrum beta lactamases can hydrolyze broader range of beta lactam antibiotics that contain an oxyimino-group such as oxyimino-cephalosporins (e.g., ceftazidime, ceftriaxone, cefotaxime) as well as oxyimino-monobactam (aztreonam) (Gupta, 2007; Livermore *et al.,* 2007) than the simple parent â-lactamases from which they are derived. It is third generation cephalosporins that causes mutations in TEM-1, TEM-2 and SHV-I enzymes that leads to generate ESBLs (Nathisuwan *et al.,* 2001; Ayyagari and Bhargava, 2001). Thus, developing ESBLs is leading cause of resistance to cephalosporins.

In last four decades a variable and rising incidence and prevalence of ESBLs has been reported ESBL-producing *Klebsiella spp*. and *E. coli* worldwide i.e. Germany (Knothe *et al.,* 1983), France (Sirot *et al.,* 1987; Philippon *et al.,* 1989), England (Du Bois *et al.,* 1995) USA (Saurina *et al.,* 2000; Mathai *et al.,* 2001; Winokur *et al.,* 2001), Canada (Cordero *et al.,* 2004), Spain (Romero *et al.*, 2007), Taiwan (Kuo *et al.*, 2007), Turkey (Hoþoðlu *et al.*, 2007), Algeria (Messai *et al.,* 2008), China (Xiong *et al.*, 2002) etc. Researchers have recorded presence of ESBL positive *E coli* from Hospital intensive care units (Shakya *et al*., 2017; Singh *et al*., 2016), farm livestock (Dahms *et al.,* 2015) and from house hold latrines' door handle (Erb *et al.,* 2018). It is recommended that the ESBL infected patients should be avoided to visit public settings to prevent spread among other.

Antibiotic resistance of bacterial pathogens is one of the most worldwide maltreatment to public

health care. To prevent selection and dissemination of resistance, the use of traditional antibiotics must be limited and alternative effective therapies must be sought (Wood *et al.,* 1996). To overcome this problem about 80% world populations depends on Plants or agricultural products. However, due to environmental pollution there are high level toxic residues in the agricultural products which may harm the human health (Premathilake *et al*., (2018). Thus, there is a high need of study and scientific evaluation of traditional knowledge of plants based medicines or recipes/preparations.

The licorice plant (*Glycyrrhiza glabra* Family *Leguminoceae* or *Fabaceae*) has been used in folk medicine since time immemorial. Many of the claims for the effectiveness of licorice extracts have been shown by modern science to be credible, a root component (Glycyrrhizin) being generally regarded as the major biologically-active principle (Nitalikar *et al.,* 2010). Glycyrrhizin is widely used in pharmaceutical and confectionery industries (Fenwick *et al.,* 1990).

Despite ESBL production antibiotic resistant bacteria can damage mitochondria and then damaged mitochondria release large amount of free radicals. These free radicals are able to suppress the immune system. Free radical stress leads to tissue damage and may eventually lead to death of patient. Several studies are ongoing worldwide to find natural antioxidants of plant origin. *Glycyrrhiza glabra* has also been studied for same. Further, we initiated this study to evaluate specific antioxidants and antibacterial activity against gram negative pathogens especially, *Escherichia coli.*

MATERIALS AND METHODS

Collection of samples

The roots of *Glycyrrhiza glabra* were purchased from local market of Agra, India. The same were cross-identified by their vernacular names and later validated at the Department of Botany, School of Life Sciences, Dr. Bhim Rao Ambedkar University, Agra. Voucher specimens (accession number Bot.0001/2012/0010) were deposited for future reference in the herbarium of same department.

Processing of samples

The withered roots (300 g) of *Glycyrrhiza glabra* (Family: Fabaceae) were washed vigorously with tap water. After that, roots were placed in shade to dry for 10-15 days. All dried material was chopped into small fragments. They were then reduced into a fine powder with a kitchen grinder. The powder could then pass through a sieve of pore size 0.5 mm. The part left in sieve was grinded again and again till we get all material in coarse powder.

Preparation of methanol extract

Powdered samples (30 gm) were extracted at 65^{o}C with methanol (400 ml) for 72 h (25 cycles) using Soxhlet apparatus to make methnol extracts. The thimble was carefully filled in with keeping at least 1 cm gap between the sample and the top of the thimble. Weight of the filled thimble was 30.2 g. Finally, the extract was filtered and concentrated in oven at 40^{o}C ± 5°C under atmospheric pressure, to obtain semisolid paste, after drying; they were weighed in order to know the amount of extract of plant sample and percentage yield. The same procedure of extract preparation was repeated with the remaining powder for two times more.

Preparation of aqueous extract

The aqueous extract of *G. glabra* was obtained by using a hot water extraction method. The dried powdered sample (30 gm) was mixed in 300ml distilled water at 70°C in a marked flat bottom flask. The distilled water was evaporated during this incubation. A known quantity (in volume) of distilled water was added repeatedly after evaporation up to the mark. This step was repeated till 5L of water was utilized for resulting extract. Then it was filtered using Whatman filter paper (No. 1) and concentrated in oven at 45^{o}C under atmospheric pressure to give a semisolid paste. The % yield of plant extract was calculated. Both extracts were stored at 4^{o}C till further use.

Phytochemical analysis

The presence of phytochemicals was screened. Alkaloids, saponins, tannins (5% ferric chloride), terpenoids (2, 4-dintrophenyl hydrazine) and steroids (Liebermann-Burchard test) were estimated according to the methods described by Edeoga *et al.* (2005) and Roy *et al.,* (2011).

Detection of Alkaloids

Protocol was adopted from Edeoga *et al.(*2005); Roy *et al. (*2011).

Dragendorff's test

A few drops of Dragendorff's reagent (0.4 g of bismuth subnitrate in 10 ml HCl 12 N; mixed with 5 g of potassium iodide in 50 ml distilled water) was added to 1ml solution filtrate obtained from 0.5 g of the extract and stirred with 5 ml of 1% aqueous HCl on steam bath. Orange precipitation indicated the presence of alkaloids.

Wagner's test

A fraction of the extract was treated with Wagner's reagent (1.27 g of iodine and 2 g of potassium iodide in 100 ml distilled water). The formation of reddish brown coloured precipitate indicates presence of alkaloid.

Detection of Steroids

Protocol was adopted from Edeoga *et al.(* 2005); Roy *et al. (*2011).

Liebermann-Burchard test

Acetic anhydride (2 ml) was added to 0.5 g methanol extracts in 2 ml of H_2SO_4. The change in color from violet to blue or green indicated the presence of steroids.

Detection of Terpenoids

Protocol was adopted from Edeoga *et al.(*2005); Roy *et al.(*2011).

Salkowski test

5 ml of extract were mixed in 2 ml of chloroform and layered over 3 ml of concentrated H_2SO_4. A reddish-brown color of the interface demonstrated the presence of terpenoids.

Detection of Tannins

Protocol was adopted from Edeoga *et al. (*2005); Roy *et al. (*2011).

About 0.5 g of the dried powdered sample was boiled in 20 ml of water and then filtered. A few drops of 0.1% ferric chloride was added to the filtrate and observed for brownish green or a blue-black colouration which suggested the presence of tannins.

Detection of Saponins

Protocol was adopted from Edeoga *et al.(* 2005); Roy *et al. (*2011).

Frothing test

The frothing test was used to check the presence of saponins. 2 gm of the extract was mixed in 20 ml of distilled water, boiled in a water bath, and filtered. 10 ml of the filtrate was taken aside, and an additional 5 ml of distilled water added and shaken vigorously to generate a stable, persistent froth. Froth formation indicated the presence of saponins.

Detection of Flavonoid

Protocol was adopted from Edeoga *et al.(* 2005); Roy *et al. (*2011).

Alkaline reagent test

Crude extract was mixed with 2 ml of 2% NaOH solution. An intense yellow colour was formed which turned colourless on addition of few drops of diluted acid indicates presence of flavonoids.

Detection of Carbohydrates

Protocol was adopted from Edeoga *et al.(* 2005); Roy *et al. (*2011).

Molish Test

In 2 ml of extract solution 3 drops of á-napthol solution (0.5 gm á-napthol in 100ml ethanol) was added, then 2 ml of conc H_2SO_4 poured along the side of the test-tube. Reddish purple ring at the interface indicates carbohydrates.

Detection of Protein

Protocol was adopted from Edeoga *et al.(* 2005); Roy *et al. (*2011).

Ninhydrin Test

Crude extract was boiled with 2 ml of 0.2% Ninhydrin solution, development of violet color indicated the presence of proteins.

HPLC analysis

The methanol & aqueous extracts were prepared for High Performance Liquid Chromatography (HPLC) by dissolving completely dried samples in HPLC grade methanol at 10 mg/ml

concentration. All samples were filtered through 0.45µm (Millipore, Bedford, MA) filter. Standard (1.2 mg/5ml), was prepared in HPLC grade methanol. Analysis was performed with waters HPLC system (Waters, Miliford, MA), equipped with 515 binary gradient pumps, 717 plus injector, 2996 PDA detector and Empower software (version 3.0). Extracts were separated on RP-18 column (4.0 X 250mm, 5µm, merck). The mobile phase consisted of acetonitrile – 2% acetic acid (40:60) delivered at a flow rate of 0.8 ml/min. The column temperature was maintained at 30° C. The UV chromatograms were recorded at 254 nm. 10 µL of the methanol & aqueous extract was injected.

Antioxidant assays

In this phase, after testing the phytochemicals, in order to check the other medicinal value of *G. glabra*, the antioxidant property of the candidate plant was analysed. Based on the results of phase I, the root of *G. glabra* was taken for further study and the antioxidants were analyzed in them. The methodology adopted for analyzing these parameters is given below.

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

The effect of aqueous extract on DPPH radicals was estimated according to the method of Molyneux (2004) and Roy *et. al.*(2011), with minor modifications. The aqueous extract and methanol was lyophilised and dilutions from 1.4 mg/ml to 3.0 mg/ml prepared in ethanol and methanol respectively. Again this solution was diluted to make working solution by adding 100 µl of extract with 1.9ml ethanol or Methanol respectively. Then, One millilitre (300 mM) of DPPH solution was mixed with 1.0 ml of working extract solution. The reaction mixture was vortex-mixed thoroughly and incubated at room temperature in the dark for 20 min. Reduction in the absorbance of the mixture was measured at 517 nm using ascorbic acid as a control. Scavenging of DPPH radicals by the extract was calculated. The half maximal inhibitory concentration (IC_{50}) values denoted the concentration of sample required to scavenge 50% of DPPH free radicals.

Reduction in the absorbance of the mixture was measured as mentioned above for aqueous extract using ascorbic acid as a control. To cover a wide range of concentration doubling dilution of aqueous ascorbic acid solution (10mg/ml) was prepared.

The reactions were repeated three times for each dilution, and then take the mean value for % of inhibition DPPH scavenging activity.

Ferric reducing antioxidant power (FRAP) assay

Method of Ferreira *et al.* (2007) and Roy *et al.* (2011) was adopted with modification to estimate reducing power in root. Briefly, crude extract was mixed with phosphate buffer (0.2 M, pH 6.6) to make working solution of different dilutions (1.4mg/ml to 3.0 mg/ml). Then working solutions (2.5 ml) was added with 1% potassium ferricyanide (2.5 ml). After 30 minute incubation at 50°C, 10% trichloroacetic acid (2.5 ml) was added and the mixture was centrifuged at 2000 × g for 10 min. Then, supernatant (1.5 ml) was mixed with distilled water (1.5 ml) and a freshly prepared 0.1% $FeCl_3$ solution (0.3 ml). After 10 minute incubation, absorbance were read at 700 nm using a PC based UV/visible light spectrophotometer, 2201 (Systronics Inc., India). The iron (III) reducing activity determination was performed in triplicate.

Evaluation of antibacterial activity of plant root extract

ESBL positivity of *E coli* was defined based on interpretation of results of Kirby-Bauer disc diffusion (1966) and recommendation by Clinical and Laboratory Standard Institute (CLSI) guidelines, 2012. The disc diffusion method adopted by Kirby-Bauer was applied to test the antibacterial activity of plant root extracts. TEM SHV and CTXM positive strain of *Escherichia coli* was grown on different culture media to performing antibacterial assay and the maintenance of strains. Nutrient broth (NB), Nutrient agar media (NA) and Muller Hinton agar media (MHA) were used in the study. Strains were activated by inoculating a loopful culture in the nutrient broth (30 ml) incubated for 4 hours to maintain McFarland standard turbidity (10^8cells/ml). Then 0.1ml of inoculums was inoculated on MHA & NA and spread uniformly using sterile cotton swab. Simultaneously, various dilution (20mg/ml, 10mg/ml, 5mg/ml and 2.5 mg/ml) of root extracts were

obtained using doubling dilution of 20mg root extracts rehydrated in distilled water. Of these 40 µl was introduced on the filter paper disc (6mm) and allowed to dry. Thus, disc had 0.8 mg, 0.4 mg, 0.2 mg and 0.1mg dose of extract. The dried impregnated discs were placed on both media. Dimethyl sulfoxide (DMSO) was used as a negative control where as cefotaxime (Taxime, 1gm, Manufactured by Alkem India pvt Ltd, reconstituted in 5ml distilled water to make a stock of 200mg/ml) was used as a positive control. Plates were incubated at 37^0C for 24 hours. Antimicrobial activity was expressed as the mean diameter of zone of inhibition (mm) around the disc as measured with vernier calliper. For positive control antibiotic disc cefotaxime was placed on similarly prepared plates with the same culture. The assay was done in triplicates and plates were incubated for 24 hours at 37°C for antibacterial activity. The mean of three readings of diameter of zone of inhibition were presented. The *E coli* strain was also positive for TEM, SHV and CTX-M genes as per results of specific PCR.

MIC/MBC Estimation

Macro Broth dilution method (Clinical and Laboratory Standard Institute (CLSI) guidelines, 2012) was used to determine the MIC/MBC of both extracts. Serially diluted extracts (3.125, 6.25, 12.5, 25 & 50 mg/ml) was impregnated in 5 test tubes separately. Thereafter, 200 µl of fresh broth culture was inoculated in each and left to grown at 37^oC for 24-36 hrs. The Minimum Inhibitory Concentration (MIC) value was noted from the tube with no visible growth but lowest concentration of extract. All the test tube without visible growth was then streaked over Nutrient agar plate. Test tube concentration belong to a non visible growth on Nutrient agar plate after incubation for 24-36 hrs at 37^oC was considered to be having Minimum Bactericidal Concentration (MBC).

Statistical analysis

All data were expressed as mean ± standard deviation of three independent replicates. The results were statistically analyzed by analysis of variance (ANOVA) and significant differences among means from triplicate analyses at ($P < 0.05$) were determined by Bonferroni multiple comparisons using the GraphPad Prism (ver 5.0).

RESULTS

Phytochemical screening

The yield obtained from aqueous extract (71.42%) was higher than methanol extract (65.09%) of *G. glabra*. Phytochmical analysis of both extracts of *G. glabra* showed presence of terpenoids, sugars, saponins, tannins, and flavonoids (Table1).

These peaks were at different retention time (rt). The contents of the *G. glabra* were investigated using standard curve for validated HPLC method. The analytical results showed that the amount of glycirhizin (rt 5.9 min) in the methanol and aqueous extract was 0.1991 mg/ml and 0.1676 mg/ml, respectively.

On comparing the chromatograms, it was observed that few peaks were exclusive to aqueous extract and few were exclusive to methanol extract.

Identification and quantification of *glycirhizin* in both extracts using HPLC

The HPLC chromatogram of the methanol extract (Figure 1) and aqueous extract (Figure 2) has shown 10 and 9 well defined chromatographic peaks, respectively.

Screening of antioxidant activity

DPPH assay (Free Radical scavenging activity)

The dose-response curves of DPPH radical scavenging activity of the methanol & aqueous extract of roots of *G. glabra* were compared with those of ascorbic acid (Figure 3 & 4). The decrease in absorbance of DPPH radical caused by antioxidants because of the reaction between antioxidant molecules and radical progress which results in the scavenging of the radical by hydrogen donation. It is visually noticeable as a change in colour from purple to yellow. *Glycyrrhiza glabra* exhibited a comparable anti oxidant activity with that of standard ascorbic acid at varying concentration tested. There was a dose dependant increase in the percentage antioxidant. The scavenging activity of control (ascorbic acid) was comparable to methanol and aqueous extracts. The obtained IC_{50} values of methanol and aqueous extract of roots were 1.372 ± 0.36 mg/ml and 1.338 ± 0.34 mg/ml, respectively and for ascorbic acid 1.482 ± 0.11 mg/ml.

Table 1: The presences of Phytochemicals in root of *Glycyrrhiza glabra*

Phytochemicals	Methanol extract	Aqueous extract
Terpenoids	+	+
Tannins	+	+
Alkaloids- Dragendorff's reagent Wagner's reagent	—	—
Flavonoids	+	+
Saponins	+	+
Carbohydrates	+	+
Proteins	-	-

Note- (+) Present, (-) Absent

Table 2a: Evaluation of antibacterial activity of aqueous extract and methanol extract of *Glycyrrhiza glabra* against *E coli* strain

S.No	Extract	20 mg/ml	10 mg/ml	5 mg/ml	2.5 mg/ml
1	*Aqueous Extract*	15 mm	14.5 mm	13 mm	10 mm
2	*Methanol extract*	20 mm	12.9 mm	10.6 mm	7.15 mm

Table 2b: Evaluation of cefotaxime susceptibility against *E coli* strain as per CLSI 2012 recommendation

S.No	*Medicine*	40 µg/ml	30 µg/ml	20 µg/ml	10 µg/ml
1	*Cefotaxime*	19.75 mm	16.25 mm	17.00 mm	16.25 mm

Note: Zone of inhibition e"22 mm for 30µg/ml dose of cefotaxime is suggestive of resistance strain (CLSI 2012 Guidelines).

Table 3: MIC and MBC of methanol and aqueous root extract of *Glycyrrhiza glabra*

		E. coli
methanol extract	MIC (mg/ml)	12.5
	MBC (mg/ml)	12.5
aqueous extract	MIC (mg/ml)	6.25
	MBC (mg/ml)	6.25

MIC & MBC of cefotaxime against *E. coli* were 4ì g/ml and 13ì g/ml, respectively.

Note: MIC>2ìg/ml for cefotaxime against *E. coli* is suggestive of ESBL production.

Table 4: Evaluation of synergistic and alone antibacterial activity of glycirhhizin and cefotaxime against *E coli* strain

S. No.	Concentration(µg/ml)	Zone of Inhibition(mm)		
	Glycirhhizin	Cefotaxime	Glycirhhizin + Cefotaxime	
1	10	8.2	16	16.2
2	20	12.2	17	16.8
3	30	14.5	16.3	17.4
4	40	14.5	18.6	18.2

FRAP (Ferric Reducing Antioxidant Power) assay

In vitro ferric reducing power assay method is based on the principle that substances, which have reduction potential, react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm. FRAP activity of the methanol & aqueous extracts and standards increases with the increase in amount of standard and sample concentrations (Fig 5).

Screening and evaluation of antibacterial activity

The antibacterial properties of the *Glycyrrhiza glabra* roots were checked by Disc diffusion method. Methanol and aqueous extracts were significantly active against *E. coli* (Table 2a) as they showed Zone of inhibition (in mm) in comparison to control. Cefotaxime was used as positive control (Table 2b). In the present study the MIC/MBC values were recorded same (Table 3).

The major compound Glycirhhizin of *G. glabra* was used to check synergistic antibacterial activity with cefotaxime (Table 4). The half dose of cefotaxime and glycirhhizin was applied in synergistic experiments of disc diffusion.

DISCUSSION

The major side effect and also the resistance to conventional medicines has increases the interests of using the natural plant extracts as antioxidant and antimicrobial source against oxidation and microbial growth to protect human health. These reasons as well as the emergence of novel microbial infections and oxidation damages are behind the recent rise in work to isolate an antioxidant & antimicrobial drugs from plants (Marchese and Shito, 2001) or other natural sources. Indigenous evidences indicated a well-known role of the plants in providing better health and nutritional security at an inexpensive price to human beings and animals due to their antioxidants and other nutrients (Momin *et al.,* 2018). In present study we addressed the antioxidants and antibacterial effects of *G. glabra* root purchased from the local market. The root of *Glycyrrhiza glabra* was selected on the basis of literature about their traditional use in folk lore medicine in India and other countries.

The day by day appearance of multi-drug resistance in microbes due to ESBLs has decreased the choice of safe drugs. Recently, to suggest novel therapeutic molecule researchers explored phytochemicals. Phytochemicals are ubiquitously present in plants, and when plants are consumed as foods or medicine (decoction, tonic, syrup, etc), these phytochemicals contribute to the intake of natural antioxidants in humans as well as animals. *G. glabra* extracts have antioxidant activity that have been proved by FRAP and DPPH assays. Ascorbic acid, a well known reference of antioxidant capacity was used to determine the plant root extracts antioxidant power. The aqueous and methanol both extracts have higher antioxidant capability that is comparable to ascorbic acid. Both extracts have shown significant FRAP & DPPH activity ($P<0.05$). However mehanolic extract of *Glycyrrhiza glabra* showed better antioxidant activity than aqueous extract.

Evidences suggest that phytochemical compounds (primary and secondary antioxidants) with reducing power are electron donor and can

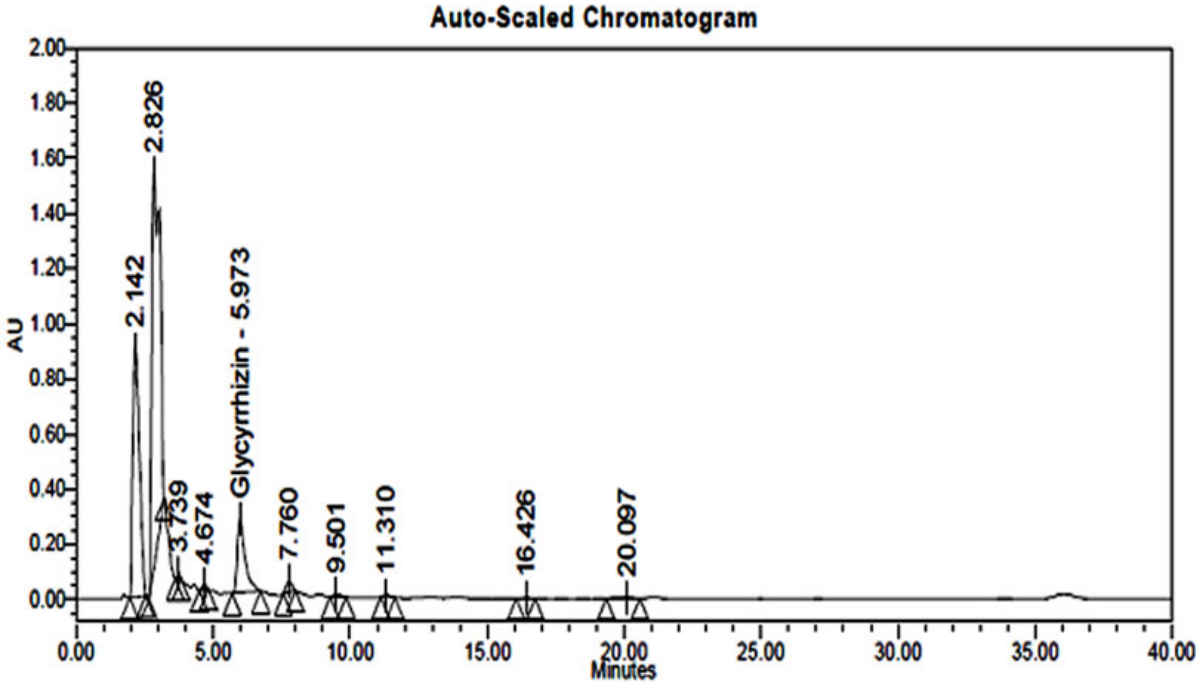

Figure 1: High performance liquid chromatographic pattern of *G glabra* Methanol extract.

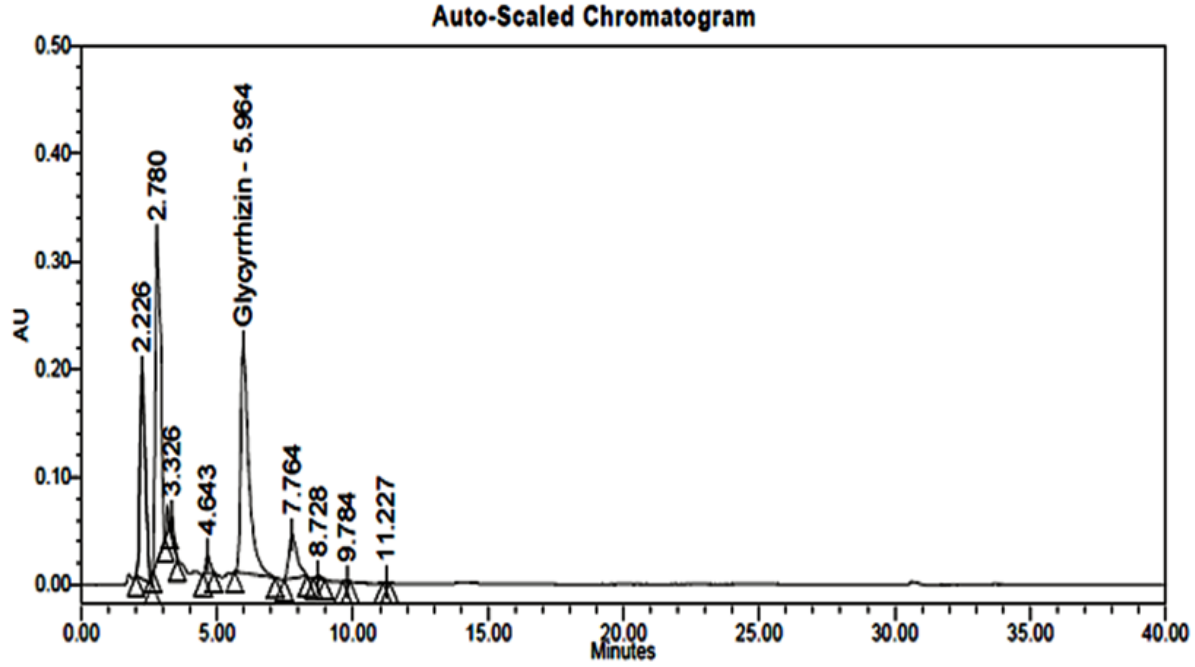

Figure 2: High performance liquid chromatographic pattern of *G glabra* aqueous extract.

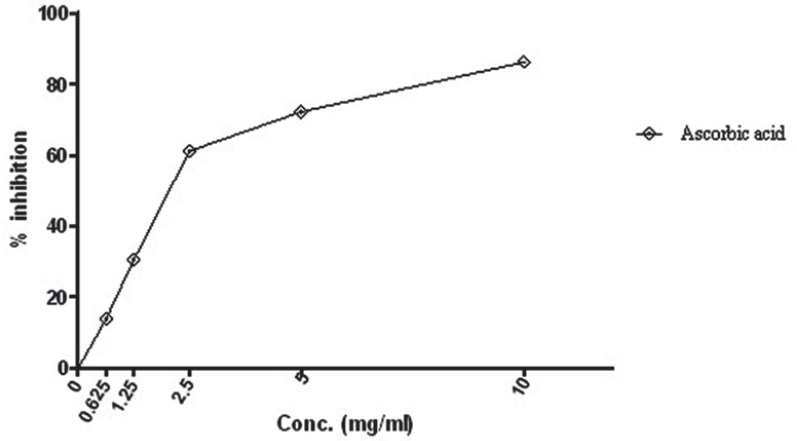

Figure 3: Percent inhibition of standard (Ascorbic acid) at various concentrations (mg/ml) in DPPH assay.

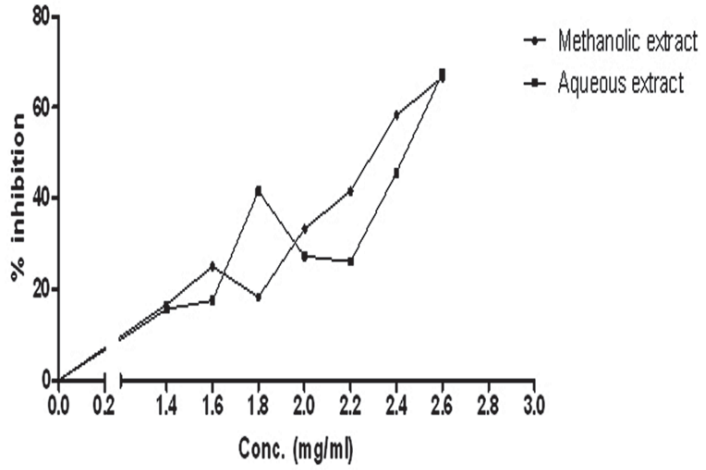

Figure 4: Percent inhibition of aqueous and methanol extract of *G. glabra* at various concentrations (mg/ml) in DPPH assay

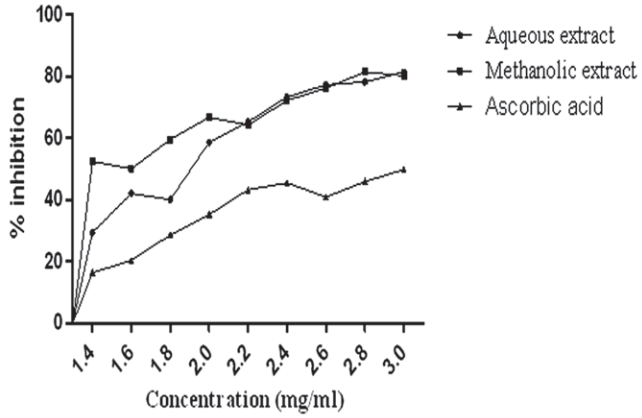

Figure 5: The percent inhibition of methanol and aqueous extract of *G. glabra* and also of ascorbic acid at various concentrations (mg/ml) in ferric reducing power assay

reduce free radicals (Chanda and Dave, 2009). In biological systems, phenolic compounds are major group of secondary metabolite that could be an important part of plant defense with supplying an antioxidant activity (Wuyts *et al.,* 2006) to plant. *Glycyrrhiza glabra* have various group of phytochemical compounds viz terpenoids, tannins, flavonoids, and saponins (Table 1). Free radical scavenging property may be one of the mechanism by which this plant is effective as a traditional medicine. It is well documented previously that most of tannins, terpinoid, & saponins are phenolic compounds and responsible for antioxidant properties of many plants. So this activity may be due to the presence of phenolic compounds tannins, terpenoids, & saponins in these extracts. These results are also in conformity with the findings of several workers viz. Roy *et al.* (2011).

An attempt has been made to identify the antibacterial activity of the methanol & aqueous extract of *Glycyrrhiza glabra* against the cefotaxime resistance *E. coli.* These initial findings show inhibitory effects against resistance *E coli* with high dose but that is also equivalent to cefotaxime. Still because of higher antioxidant potential we suggest *G glabra* could be useful in developing medicine against resistance pathogen. The antioxidant molecule improves immunity to fight against pathogens (Puertollano *et al*., 2011; Brambilla *et al*., 2008).

Furthermore, HPLC analysis indicates that 10 and 9 peaks in methanol and aqueous extracts, respectively, including glycyrrhizin. The amount of glycyrrhizin was more in methanol extract than aqueous extract. On comparing retention time, 8 peaks were seems to be common in both extract while one was present exclusively in aqueous and 2 in methanol extracts. These compounds responsible for these peaks might be responsible for variation in antimicrobial activities between two extracts. The synergistic antibacterial potential of glycyrrhizin with cefotaxime indicates that the *G glabra* could be an important supplement to enhance the antibacterial effect of conventional medicine in drug resistances cases (Table 4).

This study on *G. glabra* root determined the therapeutic potential of this plant that can be used to discover bioactive natural products that may serve as lead for development of new pharmaceuticals that the address hitherto unmet the therapeutic needs. This plant have established edible role in Ayurveda hence *in vivo* clinical testing can be perform to confirm these *in vitro* results.

CONCLUSIONS

Glycyrrhiza glabra is a significant choice to inhibit the infections caused by ESBL positive Gram negative pathogens, by presence of potent novel therapeutic and antioxidant molecules. The *G glabra* extract is also useful to enhance the antibacterial activity on synergistic uses.

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Phytochemical composition and anti-oxidant properties of *Dialium ovoideum thwaites* (Gal Siyambala) leaves

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ABSTRACT

Dialium ovoideum thwaites(Fabaceae) is an endemic plant to Sri Lanka, found in the semi-dry zone of the country. The various parts of the plant have being used for treating many health disorders in the traditional medicinal system of Sri Lanka. The aim of this study was qualitative and quantitative determination phytochemicals and evaluation of anti-oxidant properties of the leaves. Methanolic extract of D. thwaites leaves was prepared by macerating and then subjected to phytochemical screening using standard procedures. The results of phytochemical screening showed the presence of alkaloids, flavonoids, saponins, steroids, glycosides, tannins and coumarins. Quantitative determination was done for alkaloids, flavonoids and saponins using gravimetric method, and for tannins using colorimetetric method. It was found that the leaves contains 2.05% (w/w) of alkaloids, 3.58%(w/w) of flavonoid, 2.07 %(w/w) of saponins and 370.4 mg TAE / g of tannin. The anti-oxidant properties of leaves were evaluated as total phenolic content(TPC) using Folin-Ciocalteau reagent and colorimetric method as garlic acid equivalent, using free radical scavenging assay (DPPH assay) with ascorbic acid as standard anti-oxidant and using FRAP assay as total anti-oxidant capacity. Results indicates that total phenolic content is 189.7 mg GAE/ g,IC_{50}value for DPPH assay is131 mg/mL whereas 31.0mg/mL is for ascorbic acid standard, and the FRAP value gives as977 μ mol Fe^{2+} /g, showing higher anti-oxidant properties of leaves.

Keywords *: Dialium ovoideum thwaites*, phytochemical analysis, anti-oxidant, DPPH Assay, FRAP Assay

INTRODUCTION

Since the beginning of mankind, humans have been relying on plants to fulfill their vital requirements hence plants have played a salient role as a good source of medicine (Gurib-Fakim, 2006 and Komolafe, 2014, Sabitha Rani *et al*., 2019). In the modern medical aspects many plants or plant-based materials have gained wide attention on developing/extracting potential drug candidates especially for the treatment of non-communicable diseases such as diabetes mellitus and cancer etc (Bhowmik, 2019). According to a report of World Health Organization 80% of the world's population in developing countries still rely on plant products for their primary health care (Tanveer *et al*., 2017 and Aqil *et al*., 2010). Furthermore it has been reported that 61% of novel drugs which have been developed between 1981 and 2002 have been based on natural products and has been reported to be successful in areas of infectious diseases and cancer (Bhalodia and Shukla, 2011).

Nevertheless extent of such discoveries is not satisfactory compared to the estimated number of higher plants on earth which is about 250,000 whereas only 6% of them have been screened for their biological activity and only 15% has been analyzed phytochemically (Fabricant and Farnsworth, 2001) even though phytochemicals serve as the base for their potent medicinal activity. Antioxidant activity is one such property in which the world is keener today and plants are very popular in this aspect due to their innate ability in biosynthesizing a wide range of antioxidants which prevent or delay cell damages caused by oxidative stress (Kasote *et al*., 2015). Many of such unexplored plants including endemic and native plants are being used in different traditional medicine systems all over the world (Gülçin *et al*. 2010). Sri Lankan Ayurvedic system is such a traditional system which has a history of about 2500 years and is based on a series of prescriptions handed down from generation to generation (De Alwis, L. 1997). Ayurveda uses about 550 to 700 species out of over 3000 vascular plant species present and a quarter of which is endemic to Sri Lanka (De Alwis, 1997).

Dialium ovoideum thwaites (*Fabaceae,* local name; Gal siyambala) is an endemic plant to Sri Lanka used in Ayurvedic system for different applications such as treating skin infections, as an antidote to treat snake bites etc. Since no adequate scientific study has been reported so far on *D. thwaites*, this study was aimed at investigating the phytochemical profile and antioxidant capacity of leaves of *D. thwaites*. Furthermore while ethnomedicinal plants are being scientifically investigated, it is equally important to produce ready to use products by incorporating them in order to give the maximum benefit to the society. Thus the ultimate objective of this study was to produce such a value added product by making use presence of important phytochemicals and anti-oxidants.

MATERIALS AND METHODS

Chemicals and Instruments

The solvent, methanol was distilled prior to maceration while all other chemicals such as chemicals for phytochemical screening, Folin-Ciocalteu's (FC) reagent, gallic acid, tannic acid, DPPH, $FeSO_4.7H_2O$, TPTZ were analytical grade thus were used without any further purifications. For vacuum evaporation the rotary evaporator,model Heidolph WB 2000 was used while UV spectrophotometer, model SHIMADZU UV-1601was used for the spectrometric analysis.

Plant Material

The leaves of *D. thwaites* were collected from Wellawaya area, Monaragala district of Sri Lanka and were authenticated. The leaves were washed and blot dried followed by standing for 21 days in shade for complete drying as the leaves are watery in nature. The dried leaves were then ground to fine powder using a blender and stored in sealed zip-lock bags at 4^0C until usage.

Extraction of phytochemicals

For the preparation of extract, 160 g of dried powder was macerated with 600 ml of methanol for 4 days with frequent agitation. Then the macerated solution was filtered and concentrated using the rotary evaporator to obtain a solid methanolic crude.

Phytochemical screening

Screening for Alkaloids

Mayer's, Wagner's and Dragendroff's Tests - About 5 g of powdered plant material was mixed with 8 mL of 1% HCl and was boiled in a water bath for 5 minutes. The solution was cooled and filtered, the filtrate was tested with few drops of Mayer's, Wagner's and Dragendroff's reagents (Bulugahapitiya, 2013; Ezeonu and Ejikeme, 2016; Abulude, 2007). For the conformation, 6 g of powdered plant material moistened with water and mixed with 1 g of $Ca(OH)_2$. The paste was mixed well with 5.0 ml of diethyl ether followed by evaporating of ether. The residue was mixed with 5.0 ml of 1% H_2SO_4, filtered and the filtrate was tested Draggendroff's reagent.

Screening for Flavonoids

Alkaline reagent Test - About 500 mg of methanolic crude was dissolved in 2 ml of MeOH. Few drops of 10% NaOHwas added followed by few drops of 10% HCl until the colour changed to colourless (Bulugahapitiya, 2013).

Lead acetate Test - About 500 mg of dried powder was mixed with 2 ml of MeOH and few drops of 1% $Pb(CH_3COO)_2$ (Bulugahapitiya, 2013).

Screening for Tannins

Ferric chloride Test - About 100 mg of dried powder was mixed with 2 ml of MeOH and 1.0 ml of 2% $FeCl_3$ was added. (Bulugahapitiya, 2013) Alternatively, about 0.15 g of methanolic extract was mixed with 30.0 ml of distilled water and was boiled for 10 minutes. The solution was filtered and 5.0 mL of the filtrate was mixed with few drops of 0.1% $FeCl_3$ solution (Ezeonu and Ejikeme, 2016).

Screening for Saponins

Froth Test - About 2 g of powdered plant material was mixed with 15.0 ml of distilled water. The mixture was boiled and filtered; the filtrate was mixed with 5.0 mL of distilled water and was shaken vigorously. Then the formed froth was mixed with 3 drops of olive oil and was again shaken vigorously (Bulugahapitiya, 2013).

Screening for Terpenoids

In the first method about 2 g of powdered plant material was defatted with petroleum ether and the

residue was extracted with 10.0 ml of $CHCl_3$followed by drying. To 5.0 ml of the above extract, 0.25 ml of acetic anhydride followed by 2 drops of conc. H_2SO_4were added. In the second method, about 1 g of dried plant material was mixed with 5 drops of $Cu(CH_3COO)_2$ solution (Bulugahapitiya, 2013).

Screening for Glycosides

Keller-killani Test- About 1 g of powdered plant material dissolved in 3 ml of glacial acetic acid and few drops of 5% $FeCl_3$ was added. The mixture was poured in to a test tube containing 2.0 ml of conc. H_2SO_4 (Bulugahapitiya, 2013). Alternatively in to 1 g of powdered plant material, 5.0 ml of $CHCl_3$ were added followed by 5.0 ml of 10% NH_3 (Ezeonu and Ejikeme, 2016.

Screening for Coumarins

About 1 g of powdered plant material was mixed with 2 ml of MeOH. The mouth of the test tube was covered with a filter paper soaked in 1 N NaOH. The tube was placed in a boiling water bath for few minutes. The filter paper was then removed and immediately observed under UV light (Bulugahapitiya, 2013).

Screening for Steroids

About 1 g of powdered plant material mixed with 20.0 ml of ethanol, covered and was allowed to stand for 2 hours. The solution was filtered and the second mixture was prepared by mixing 2.0 ml of acetic anhydride with 2.0 ml of conc. H_2SO_4. 5.0 ml of the sample extract prepared above was mixed with the prepared acid solution (Bulugahapitiya, 2013;Ezeonu and Ejikeme, 2016).

Quantitative Determination of Phytochemicals

Quantification of alkaloids

1g of powdered plant material was extracted into 10% acetic acid in EtOH, filtered and concentrated. 10 ml of Conc. NH_3added and filtered after 24h, dried at 40^0C until constant weight. The percentage alkaloids was calculated. (Ezeonu and Ejikeme, 2016)

Quantification of saponins

1g of powdered plant material was extracted into 20% aqueous of ethanol. Resulting solution was heated in a water bath at 55°C with constant stirring for 4 hours, non-polar matter was extracted off with diethyl ether. The solution was dissolved in 12.0 mL of n-butanol, after evaporating n-butaol, the saponin was weighted out (Ezeonu and Ejikeme, 2016).

Quantification of flavanoids

1g of powdered plant material was first extracted off with 20.0 ml of 80% methanol, after discarding the supernatant, the residue was extracted with ethanol. After evaporating ethanol, extracted flavonoids was dried until constant weight (Ezeonu and Ejikeme, 2016).

Quantification of tannin

An amount of 0.8967 g of methanolic crude was extracted into 10.0 ml of PET ether, 10.0 mL of acetone: water (7:3 v/v). Filtration followed by evaporation of excess solvent, it was dissolved in 25.00 ml of MeOH and methanolic crude was prepared. The stock solution (15,000 µg/ml) of tannic acid and then the concentration series was prepared to measure the absorbance at 725 nm using the UV spectrophotometer. The test solution prepared using 1.00 ml of extract, 1.00 ml of 7.5% Na_2CO_3, 0.5 ml of FC reagent and 7.50 mL of distilled water. Absorbance was measured at same wave length (Ezeonu and Ejikeme, 2016).

Evaluation of anti-oxidant capacity

Determination of total phenolic content (TPC)

Total phenol content was determined using Folin-Ciocalteau reagent and following colorimetric method. Amount of 0.3 ppm plant extract and 200 ppm standard gallic acid stock solution was prepared in MeOH followed by solution series of 20, 40, 60, 80, and 100 ppm. Folin-Ciocalteau reagent was used for complexation (2.50 ml) and 80% MeOH was used as the blank. After incubation period of 1 h at room temperature, the absorbance was measured at 765 nm using the UV spectrophotometer (Valko *et al*., 2007).

DPPH assay

DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging assay was performed using the methods reported with slight modifications (Alam *et al.,*

2013*)*. The stock solution of plant extract of 10,000 ppm and ascorbic acid of 1000 ppm was prepared in MeOH. For the test 100 µL of the extract with different concentration, 3.90 ml of DPPH solution were mixed. After 30 minutes of incubation at room temperature, the absorbance was measured at 517 nm using the UV spectrophotometer. The measurements were triplicated and the antioxidant activity was measured as percentage inhibition of DPPH as described below where A_0 and A are the absorbance of the control and the sample respectively. The IC_{50} values were obtained by plotting the percentage inhibition vs concentration.

$$\text{Percentage inhibition} = \frac{A0 - A \times 100\%}{A0}$$

FRAP assay

The Ferric reducing anti-oxidant power (FRAP) assay was performed which assessed the reduction of ferric tripyridyltriazine (Fe (III)-TPTZ) complex to ferrous tripyridyltriazine (Fe (II)-TPTZ) by measuring the absorbance at 593 nm. A 1200 ppm $FeSO_4.7H_2O$ stock solution and a dilution series was prepared in water while the same plant extract series above was used. For the test solution 100 µL of extract or standard, 3.00 mL of working FRAP reagent were mixed. Absorbance was measured after 30 minutes of incubation at 37^0C **(**Abulude, 2007).

Results and Discussion

The methanolic extract of *D. thwaites* showed the presence of eight common phytochemicals such as alkaloids, flavonoids, tannins, saponins, steroids, glycosides, coumarins and terpenoids in the leaves. The quantification gave the amounts given in the table 1 below. Accordingly leaves contains significant amount of alkaloids, flavonoids and saponins which can be directly related to many medicinal values of *D. thwaites* (Chew *et al*., 2011).

Table 1.: Quantitative analysis of phytochemicals for methanolic extract

Phytochemical class	Quantity
Alkaloids (w/w)%	2.05
Flavanoids (w/w)%	3.58
Saponins (w/w)%	2.07
Tannins mg TAE/g	370.40

The evaluating of anti-oxidant capacity of leaves was done as total phenolic content, DPPH assay and FRAP assay. The total phenolic content (TPC) was expressed in gallic acid equivalents which was 189.7 mg GAE/g (Figure 1), which was relatively a higher amount. This test is based on the oxidation of phenolic groups by phosphomolybdic and phosphotungstic acids (Folin-Ciocaltue) and yielding a blue colour with a broad maximum absorption at 765 nm, where gallic acid used as the reference. Phenolic compounds are known to act as anti-oxidants and hence it can quench the harmful free radicals generated in the cells via oxidizing of phenolic group of the substance. This property is attributed to the ability of acting against non-communicable diseases (NCDs) as there is a strong correlation between harmful free radicals and NCDs (Chew *et al*., 2011; Kukula and Widelski, 2017).

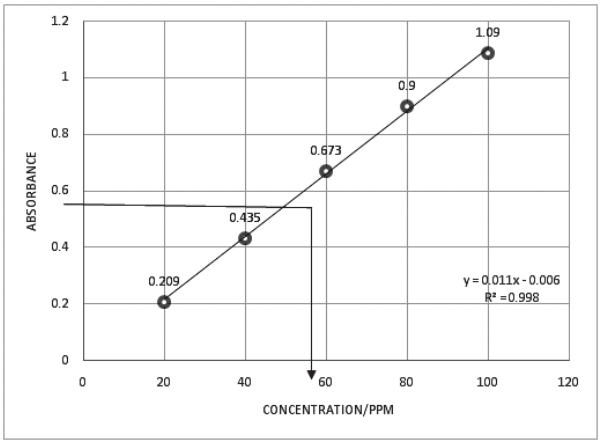

Figure 1. Standard Gallic acid curve for TPC determination

Free radical scavenging capacity is a good measurement for the ability quenching harmful free radicals generated in the cells. The DPPH assay measures the free radical scavenging ability of plant substances and also it is a method of measuring reducing power of plant compounds, it is expressed as IC_{50} value (antioxidant concentration needed to scavenge 50 % of DPPH radical). In this assay, antioxidants react with DPPH radical, forming reduced form of DPPH and the intensity of the resulting colour is proportional to the remaining concentration of DPPH after reaction with the antioxidant. The results of DPPH assay of this study is given in figure 2 and the IC_{50} values were 131.0 ppm and 31.0 ppm for leaves of *D. thwaites* and standard (ascorbic acid) respectively, implying that higher anti-oxidant capacity of leaves. Anti-oxidant capacity depends on the maturity stage of the plant (Dudareva *et al*., 2004).

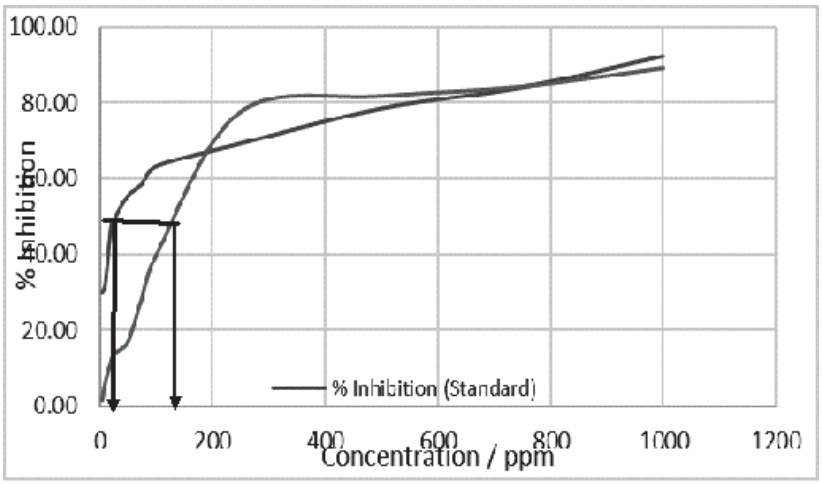

Figure 2. % Inhibition Vs concentration plot for leaves of *D. thwaites*

The FRAP value in Ferric Reducing Antioxidant Power (FRAP) assay was calculated using the standard curve drawn (figure 3) and the value was 977 µ mol Fe^{2+} /g.This further supports the anti-oxidant capacities given in DPPH assay

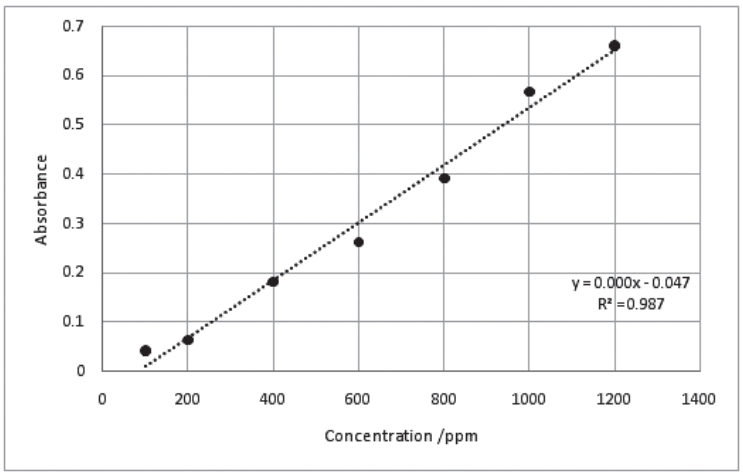

Figure 3. $FeSO_4.7H_2O$ standard curve for FRAP assay

As of *D. thwaites* is an endemic plant to Sri Lanka and it has long uses in traditional system of medicine and folk medicine in the country, the medicinal and health care properties of *D. thwaites* leaves can be scientifically attributed to presence of diverse phytochemicals along with the highest anti-oxidant capacity of the leaves.

Conclusion

This study confirms that the leaves of *D. thwaites* are rich with diverse of phytochemicals such as alkaloids, flavonoids, tannins, saponins, steroids, coumarins, glycosides and terpenoids, and it contains significant quantities of alkaloids, flavonoids, saponinsand tannins. The leaves of *D. thwaites*possess higher anti-oxidant capacity. These results validate the medicinal properties associated with *D. thwaites* and can be developed into value added products.

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Variation of shoot regeneration capacity of cuttings of selected *Cinnamomum verum* genotypes

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ABSTRACT

Vegetative propagation is practiced for elite planting material of cinnamon, which needs extensive labour. This study is aimed to determine the shoot regeneration capacity of cuttings of three Cinnamomum verum genotypes. Cuttings were collected from one cinnamon accession from Faculty of Agriculture, University of Ruhuna and, Commercial varieties Sri Gemunu and Sri Wijaya from National Cinnamon Research and Training Center, Pallolpitiya, Sri Lanka. Perimeter of cuttings was measured to group the cuttings as 2-3 cm, 4-6 cm and 7-8 cm. Three replicates of each genotype were taken from each perimeter group for a completely randomized design (CRD). Cuttings were kept in tap water filled jam bottles. A cutting consisted with 1 to 5 nodes. The length of a cutting varied from 20-30 cm. Cuttings were placed in natural open environment at Faculty of Agriculture from May, 2019. Sri Gemunu did not produce buds for the experimental period of 8 weeks. Sri Wijaya produced one bud only from a cutting of 6-8 cm perimeter group, which did not develop in to a shoot. Cinnamon accession from Faculty of Agriculture produced buds in all three replicates of 7-8 cm perimeter group at 3-4 weeks. Bud regeneration frequency of 4-6 cm group was 2/3. All buds of above accession developed into shoots. None of the genotypes produced buds in 2-3 cm perimeter group. Above results provide an insight on genetic diversity of cinnamon, which would be useful in developing method of efficient plant material production in long run.

Keywords : Cinnamomum verum, shoot regeneration, Sri Gemunu, Sri Wijaya

INTRODUCTION

Sri Lankan cinnamon (*Cinnamomum verum*) is a unique spice in export market. Sri Lanka is the world's largest true cinnamon producer. The export volume of cinnamon from Sri Lanka was 14 692.8 Mt in year 2017 (Sri Lanka Customs, 2017). *Cinnamomum* genus in Family Lauraceae consists of about 250 species and sub-species (Mabberly, 2008). There are seven wild cinnamon species endemic to Sri Lanka, which could be used as genetic resources for crop improvement of cultivated cinnamon (Sitharan, 1984; Kumarathilake *et al*., 2010). Sri Lankan cinnamon germplasm exhibits a wide morphological and chemical diversity (Azad *et al*., 2016; Azad *et al*., 2019; Azad, 2017). Two varieties of cinnamon named *Sri Gemunu* and *Sri Wijaya* from Department of Export Agriculture, Sri Lanka (2019) are based on the oil yield and quality parameters. Plant material is selected from the mother plant having desirable characters of erect stems with smooth bark, vigorous growth, pest and disease resistance, good peeling qualities, good fragrance and taste, higher dry bark yield, higher leaf and bark oil yield and oil quality. Seed propagation is the most common propagation method for cinnamon in Sri Lanka, according to the Department of Export Agriculture, Sri Lanka (2019). Cinnamon fruits are 15 to 20 mm in length (Azad *et al*, 2019) and each fruit contains a seed. Farmers collect well-ripened fruits from desirable mother plants. Recalcitrant cinnamon seeds can be kept viable for about six months under dark conditions by mixing with sand and storing in air tight poly bags. Higher seed germination percentages could be obtained if sown immediately after harvesting (Kannan and Balakrishnan, 1967).

Protogynous dichogamy in cinnamon is a reason for the cross pollination. There are two types of cinnamon plants, named A and B in any natural population. In type A plants and type B plants, the first flowering occurs in the morning and in the afternoon respectively. Cinnamon flowers are bisexual (Joseph, 1981). The stigma is receptive

Table 1: Record of shoot initiation of three perimeter groups of the cinnamon accession collected from Faculty of Agriculture, University of Ruhuna, Sri Lanka

Perimeter above 5 cm from the base	Replicate 1	Replicate 2	Replicate 3
7-8 cm	3 red colour and 1 green colour shoots	Only one green bud	4 red colour shoots and 5 green colour buds
4-6 cm	One green shoot	5 green colour shoots and 5 green colour buds	No green colour buds or shoots
2-3 cm	No green colour buds or shoots	No green colour buds or shoots	No green colour buds or shoots

during the first opening and anthers dehiscent at the second opening. Azad *et al* (2015) reported the possibility of cross pollination by comparing the mother plants and their progenies for age independent leaf morphological characters of leaf shape, leaf base and leaf apex. None of the seedlings were 100% similar to their mother plants. New phenotypes for leaf shape and leaf base were produced. Elite plant material to mother plant cannot be obtained from seeds due to cross pollination. Such seeds result in a heterogeneous cinnamon population.

Vegetative propagation is carried out through cuttings or layering to overcome the effect of cross pollination for elite and true to type planting material. Semi hard wood stem cuttings (with 1 to 2 leaves) of 2.5- 4.0 cm in length, are planted in poly bags filled with a mixture of equal parts of top soil, cow dung, sand and coir dust. Pots are kept in propagators maintaining 100% RH to prevent water loss from cuttings. Prevention of pest damages and fungal infestations are important inside the propagator. After thirty days, cuttings with shoots are placed outside in a shady place and watered regularly until about 30 cm heights. A ring bark is removed from semi hard wood shoots for the air layering. Rooting hormones should be applied on wounded area. Moist coir dust is wrapped on it with a sheet of polythene. It takes 40-60 days for rooting. Well rooted layers can be separated and transplanted in poly bags. Azad *et al* (2019) conducted an eco-geographical survey in major cinnamon growing districts in Sri Lanka and collected stem cuttings for the establishment of a core-collection at Faculty of Agriculture, University of Ruhuna. The average shoot regeneration percentage of collected 269 accessions was 47.76% after one month of planting. Only 80% of accessions survived until field planting. A protocol for micro-propagation of cinnamon using explants of axillary buds was reported from *in-vitro* grown seedlings. An embryonic axis with half of the cotyledon portions were excised from sterilized seeds with 15% Clorox for 20 min and inoculated in half strength MS medium supplemented with (1.5 mg L^{-1}) BAP, (0.2 mg L^{-1}) IAA and activated charcoal (1.0 g L^{-1}). After 14 days of culture establishment micro stem cuttings were transferred to the full strength MS medium supplemented with (0.1 mg L^{-1}) NAA, (4.0 mg L^{-1}) BAP and activated charcoal (1.0 g L^{-1}) for root initiation. Rooted plantlets were acclimatized using coir dust as the potting medium for maximum survival of 90% (Subasinghe *et al.*, 2016). However, the available measures of vegetative propagation provide a limited number of planting material for farmers due to low shoot regeneration rate in addition to the requirement of intensive labour and long period of propagators.

Development of alternative methods of vegetative propagation is required to fulfill the heavy demand for planting material by cinnamon growers, which cannot be supplied completely due to the inability to supply for the demand. Most farmers depend on seeds, which result in a heterogeneous population leading to depletion of quality of yield. Plant tissue culture techniques for cinnamon propagation are practically not feasible due to high cost of production. Vegetative propagation, being the most common alternative

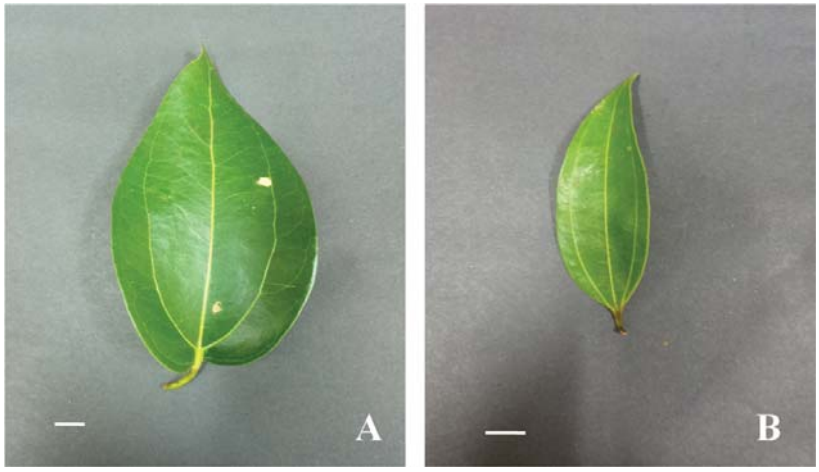

Figure 1: *Sri Gemunu* (A) and *Sri Wijaya* (B) leaves. Scale: 1 cm

Figure 2: Shoot regeneration from cinnamon cuttings in water for 4 weeks. A: 4-6 cm perimeter cuttings, C: cinnamon accession, G: *Sri Gemunu* and W: *Sri Wijaya* B: 7-8 cm perimeter cuttings, C: cinnamon accession, G: *Sri Gemunu* and W: *Sri Wijaya,* C:c_1; more than 8 cm, c_2; 7-8 cm, c_3; 4-6 cm cuttings of cinnamon accession from Faculty of Agriculture, University of Ruhuna

Figure 3: Two cuttings of the cinnamon accession collected from Faculty of Agriculture, University of Ruhuna on soil after one month of transplanting. (A) Cutting from above 8 cm perimeter group (B) Cutting from 7-8 cm perimeter group (C) Cutting of above 8 cm perimeter group at flowering

method, requires intensive labour and propagators, while only semi hard wood is effective. This study is an initial attempt on developing an alternative shoot regeneration method based on genetic potential of different cinnamon genotypes to avoid above drawbacks.

MATERIAL AND METHODS

Cuttings of one cinnamon accession were collected from Faculty of Agriculture, University of Ruhuna, Sri Lanka (GPS: 6.061445 N, 80.567595 S) and, cuttings of *Sri Gemunu* and *Sri Wijaya* were collected from National Cinnamon Research and Training Center, Pallolpitiya, Sri Lanka (GPS: 6.028302 N, 80.559563 S). Perimeter of cuttings was measured to group the cuttings as 2-3 cm, 4-6 cm and 7-8 cm. Cuttings consisted of three replicates of each genotype were taken from each perimeter group for a completely randomized design (CRD). Cuttings were kept in tap water filled jam bottles. A cutting consisted with 1 to 5 nodes. Cuttings varied from 20-30 cm in length. Cuttings were placed under shade of trees in natural open environment at Faculty of Agriculture from May, 2019. Shoot regeneration capacity of cuttings of different thicknesses from three cinnamon genotypes was determined in open environment. Two commercial varieties of *Sri Gemunu* and *Sri Wijaya* were included in order to check their potential for alternative method (Figure 1). Bud break and shoot regeneration was recorded from the cuttings. Cuttings with shoots were transplanted directly on soil for rooting.

RESULTS AND DISCUSSION

During this study, commercial variety *Sri Gemunu* did not produce buds for the experimental period of 8 weeks. *Sri Wijaya* produced one bud only from a cutting of 7-8 cm perimeter group, which did not develop in to a shoot. Above results suggests that two commercial varieties may not be suitable for the above method. Cinnamon accession from Faculty of Agriculture produced buds in all three replicates of 7-8 cm perimeter group at 3-4 weeks (Figure 2). Bud regeneration frequency of 4-6 cm group was 2/3 (Table 1). All buds of above accession developed into shoots. None of the genotypes produced buds in 2-3 cm perimeter group.

The transplanted cuttings with shoots further developed leaves at the end of the first month on soil (Figure 3; A and B). The fungal growth was observed around the basal area of the cutting edge after one month of transplanting suggesting further investigations on cinnamon and associated fungi on root development and nutrient uptake. The transplanted cutting of the 8 cm perimeter group flowered after four months. The cutting belonged to flower type A (Figure 3; C).

The age of the cinnamon accession from Faculty of Agriculture may be different from the two commercial varieties. Further, the commercial varieties were originated through vegetative propagation and the accession from the Faculty of Agriculture must be seed borne as it was grown in the wild. Therefore, the differential shoot regeneration potential among three genotypes may be due to physiological differences led by above factors. However, above results would not exclude the provision of an insight on potential genetic diversity of cinnamon, which would be useful in developing method of efficient plant material production in long run.

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Effects of pyroligneous acids (wood vinegar) produced from different wood species on vegetative growth of eggplant (*Solanum melongena* L.)

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ABSTRACT

Pyroligneous acids are natural distillations which are extracted from the slow pyrolysis technique as a by-product of charcoal production. This magical natural extract helps to replace synthetic chemicals in the form of the plant growth regulator, biocide as well as the pesticides and improves the quality and the medicinal value of fruits and vegetables. Evaluation the vegetative growth of eggplant (Solanum melongena L.) as affected by pyroligneous acids produced from different wood species are very much important. Pyroligneous acid was prepared by using the wood species of Gliricidia sepium, Cinnamomum zeylanicum, Acacia leucopholea, and Azadirachta indica. Different concentrations (0%, 0.25%, 0.50%, 0.75%, and 1.0%) of the Pyroligneous acid were applied as a foliar spray (50ml/plant) with one-week intervals when the plant is having 4-5 leaves per each plant. Eggplant (Solanum melongena L.) used as the test plant for the experiment. A pot experiment was laid out in Complete Randomized Design (CRD) with twenty treatment combinations and four replications. Treatments were applied four times with one-week interval. Numbers of leaves, plant height, stem girth, number of branches was collected as the growth parameters. Data were collected one week after the application of each treatment. Results revealed that, application of 1% pyroligneous acid for Solanum melongena plant has significantly increased 20% of the mean number of leaves, 20% of the mean plant height, 10% of stem girth, 40% of number of branches when compared to the control (0%). Among the Pyroligneous acid prepared from different species 1% concentration of the acid prepared from Gliricidia sepium acids showed the significantly highest number of branches when compared with other treatments.

Keywords : Pyroligneous acid, Growth, Wood species, Concentrations, Medicinal

INTRODUCTION

Brinjal (*Solanum melongena L.*), is one of the popular, widely cultivated and principal vegetable crops in subtropical and tropical countries of the world, including Sri Lanka. In Sri Lanka, brinjal is one of the most favoured local vegetables and is cultivated by farmers in reasonable quantities in dry and rainy seasons, while other vegetables are in lack of supply (Karunakaran *et al.*, 2010). Eggplant is the common name for a perennial plant, *Solanum melongena*, of the potato or nightshade family Solanaceae(Newworldencyclopedia.org, 2019). Some of the plant growth regulators significantly improve the fruit quality (Kavyashree *et al.,* 2018) as well as the vegetative growth. Wood vinegar consider as one of the plant growth regulators further wood vinegar/pyroligneous acids are an organic liquid mixture produced through condensing the smoke produced during the carbonization or pyrolysis of wood and its residues from processing. Acetic acid is the major compound of wood vinegar and it also contains acids, phenols, alcohols, esters, carbonyl and furans and other organic ingredients (Yatagai *et al.*, 2002, Yoshimoto, 1994, Baimark and Niamsa, 2009). Yoshimura and Hayakawa (1991) reported that wood vinegar application is promoting fruit maturation. Wood vinegar has been used in a variety of processes, such as industrial, livestock, household and agricultural products.Therefore, application of wood vinegar/ pyroligneous acid to vegetable production may help to reduce the use of both chemical pesticides and chemical fertilizers.The objective of the study is to assess the growth performances of Brinjal (*Solanum melongena* L.) as affected by different concentrations of wood vinegar/pyrolegnious acids produced by different wood species.

MATERIALS AND METHODS

The experiments were carried at the University of Colombo Institute for Agro technology and Rural Sciences, Weligatta, Hambanthota, Southern Sri Lanka. *Solanum melongena*(eggplant) variety "*Lena iri*" wasused for the experiment. Four different wood species namely *Gliricidia sepium* (Gliricidia) *Cinnamomum zeylanicum* (Cinnamon), *Acacia leucopholea* (Katuandara) and *Azadirachta indica* (Kohomba) were selected as sources of pyroligneous acids.

Extraction of pyroligneous acids

A metal barrel with 200L capacity was used as improvised equipment for thermal decomposition of the selected plant material under inert atmosphere and the resulting volatiles were passed through a condenser to collect pyroligneous acids of the plants.

Raising of *Solanum melongena*

Polythene bags were (20cm diameter and 30cm height) were filled with potting media consisting top soil, sand and compost at the ratio of 1:1:1. Twenty bags were transplanted with *Solanum melongena* variety "*Lena iri*" at the rate of two plants per pot.When the plants were 4-5 leaf stage the extracted pyroligneous acids were sprayed separately to the plants.

Experimental design and treatment application

The experimental design used for this experiment was 2 x 2 CRD factorial designs with four replicates. Purified pyroligneous acids were diluted with water to obtain 0.25%, 0.50%, 0.75% and 1% concentrations as treatments. Pyroligneous acid treatments were applied to the surface of the leaves with one-week intervals when the *Solanum melongena* plants were having four to five leaves per plant.

Wood species and pyroligneous acid concentrations act as two factors and four wood species *Gliricidia sepium* (C1), *Cinnamomum zeylanicum* (C2), *Acacia leucopholea* (C3) and *Azadirachta indica* (C4) and five concentrations of 0.00% (L1), 0.25% (L2), 0.50% (L3), 0.75% (L4), and 1.00% (L5) were used in the experiment with four replicates. All treatments were applied randomly.

Data collection

Data was collected from all plants with one-week interval after application of treatments. Number of leaves, plant height (cm), stem girth (cm), and number of branches was collected as the growth parameters.

Data analysis/Statistical method

The statistical packages of SAS used for analysis of data. Data analyzed using ANOVA and DMRT for the mean separations.

RESULTS AND DISCUSSION

Mean number of leaves

Table 1: Number of leaves of *Solanum melongena* as affected by main effect of pyroligneous acids concentrations

Treatments	Mean number ofleaves of*Solanum melongena*			
	1st week	2nd week	3rd week	4th week
L_1	5.31 ± 0.22 c	7.56 ± 0.22 d	9.81 ± 0.18 c	11.94 ± 0.20 e
L_2	6.00 ± 0.13 c	9.31 ± 0.11 c	12.06 ± 0.17 b	14.75 ± 0.17 d
L_3	6.62 ± 0.16 c	10.56 ±0.17 bc	13.31 ± 0.22 b	16.06 ± 0.19 c
L_4	8.12 ± 0.24 b	11.81 ± 0.23 b	15.06 ± 0.25 a	17.87 ± 0.24 b
L_5	9.50 ± 0.18 a	13.31 ± 0.22 a	16.31 ± 0.19 a	19.31 ± 0.18 a

** Means with the same letter(S) are not significantly different from each other according to DMRT at 5% significant level*

** The values are the means ± standard error of 80 plants in four replications.*

** Where; L_1-0% WV, L_2-0.25% WV, L_3-0.5% WV, L_4-0.75% WV, L_5-1% WV.*

Results revealed that (Table 1) the application of 1% WV (L5) and 0.75% WV (L4) concentrations of pyroligneous acids were significantly increased (P value<0.05) mean number of leaves in *Solanummelongena* plant throughout the experimental period when compared to the other concentrations of pyroligneous acids (Table 1).

Plant height

Table 2: Plant height of *Solanum melongena* as affected by main effect of pyroligneous acids concentrations

Treatments	Mean height values (cm) of *Solanummelongena*			
1st week	2nd week	3rd week	4th week	
L_1	5.31 ± 0.15 c	7.56 ± 0.16 d	9.81 ± 0.15 c	11.94 ± 0.15 e
L_2	6.00 ± 0.21 c	9.31 ± 0.20 c	12.06± 0.23 b	14.75 ± 0.23 d
L_3	6.62 ± 0.25 c	10.56± 0.22 bc	13.31± 0.20 b	16.06 ± 0.15 c
L_4	8.12 ± 0.26 b	11.81± 0.22 b	15.06± 0.20 a	17.87 ± 0.17 b
L_5	9.50 ± 0.26 a	13.31± 0.23 a	16.31± 0.23 a	19.31 ± 0.22 a

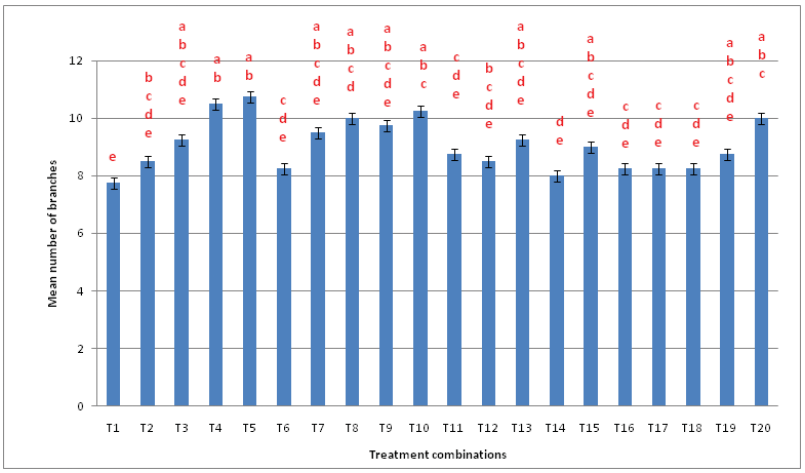
** Means with the same letter(S) are not significantly different from each other according to DMRT at 5% significant level*

** The values are the means ± standard error of 80 plants in four replications.*

** Where; L_1-0% WV, L_2-0.25% WV, L_3-0.5% WV, L_4-0.75% WV, L_5-1% WV.*

According to thetable (Table 2), 1% WV concentration of pyroligneous acids resulted a significantly increased (P value<0.05) mean plant height when compared to the other different concentrations of pyroligneous acids on *Solanum melongena* plant throughout experimental period.

Number of branches



(Figure 4.3: Mean number of branches of *Solanum melongena* as affected by treatment combinations at 4th week (Vertical lines indicate the standard error of the means)

** Means with the same letter are not significantly different from each other according to DMRT at 5% significant level*

** Where; T_1- Gliricidia sepium at 0% WV, T2-Gliricidia sepium at 0.25% WV, T3-Gliricidia sepium at 0.50% WV,T4-Gliricidia sepium at 0.75% WV,T5-Gliricidia sepium at 1.00% WVT6- Cinnamomum zeylanicum at 0% WV, T7- Cinnamomum zeylanicum at 0.25% WV,T8- Cinnamomum zeylanicum at 0.50% WV,T9- Cinnamomum zeylanicum at 0.75% WV,T10- Cinnamomum zeylanicum at 1.00% WV,T11- Acacia leucopholeaat 0% WV,T12- Acacia leucopholeaat 0.25% WV,T13- Acacia leucopholeaat 0.5% WV,T14- Acacia leucopholeaat 0.75% WV,T15- Acacia leucopholeaat 1.00% WV, T16- Azadirachta indica at 0% WV, T17- Azadirachta indica at 0.25% WV,T18- Azadirachta indica at 0.50% WV,T19- Azadirachta indica at 0.75% WV,T20- Azadirachta indica at 1.00% WV.*

The interaction effect from the treatment combination of T4 (*Gliricidia sepium* at 0.75% WV) , T5 (*Gliricidia sepium* at 1.00% WV), T8 (*T8- Cinnamomum zeylanicum at 0.50% WV*), T10 (*Cinnamomum zeylanicum at 1.00% WV*) and T20 (*Azadirachta indica at 1.00% WV*) were significantly increased (p value<0.05) mean number of branches of *Solanum melongena* plants at 4th week of the experimental period when compared to the control treatment combinations of T1 (*Gliricidia sepium at 0% WV)* (Figure 4.3). But T4(*Gliricidia sepium* at 0.75% WV) and T5 (*Gliricidia sepium* at 1.00% WV) treatment combinations did not show any significant difference (p value>0.05) each other.

Plant girth

Table 3: Mean girth (cm) of branches of *Solanum melongena* as affected by main effect of different pyroligneous acid concentrations

Treatments	Mean girth (cm) of *Solanum melongena*			
1st week	2nd week	3rd week	4th week	
L_1	1.92 ± 0.04 b	2.13 ± 0.04 c	2.36 ± 0.04 b	2.61 ± 0.04 b
L_2	1.92 ± 0.03 b	2.25 ± 0.04 c	2.46 ± 0.03 b	2.73 ± 0.03 b
L_3	1.99 ± 0.03 ab	2.32 ± 0.02 bc	2.54 ± 0.03 b	2.81 ± 0.03 b
L_4	2.05 ± 0.03 ab	2.44 ± 0.01 ab	2.76 ± 0.03 a	3.00 ± 0.03 a
L_5	2.14 ± 0.02 a	2.57 ± 0.02 a	2.85 ± 0.02 a	3.13 ± 0.02 a

** Means with the same letter(S) are not significantly different from each other according to DMRT at 5% significant level*

** The values are the means ± standard error of 80 plants in four replications.*

** Where; L_1-0% WV, L_2-0.25% WV, L_3-0.5% WV, L_4-0.75% WV, L_5-1% WV.*

Results from the above table (Table 3) exposed that the 1% WV concentrations of pyroligneous acids have shown a significantly increased (P value<0.05) mean of girth of *Solanum melongena* plants followed by the 0.75% WV concentration of pyroligneous acids when compared to 0% WV (control) concentration throughout the experimental period.Pyroligneous acids produced from different wood species were not significantly influenced (P value>0.05) on mean girth of *Solanum melongena* plants throughout the experimental period.

CONCLUSIONS

Mean number of branches of *Solanummelongena* plant were significantly increased with the application of pyroligneous acids produced by*Gliricidiasepium* wood species on 1% concentration when compared to the other interactions in later stage of the experimental period. Mean number of leaves and mean plant height of *Solanummelongena* plants were significantly increased (P value<0.05) at the1% concentration of pyroligneous acids (L5)throughout the experimental period when compared to the other concentrations of pyroligneous acids. Mean girth and mean number of branches of *Solanummelongena* plants were shown a significantly increased at the 1% concentrations of pyroligneous acids (P value<0.05) followed by the 0.75% concentration of pyroligneous acids when compared to 0% (control) concentration throughout the experimental period.

The optimization of such developmental traits thus has great potential to increase biomass and crop yield (Mathan, Bhattacharya and Ranjan, 2019).Recently, Ainsworth and Bush have suggested a need to increase source strength in order to improve yields (Ainsworth and Bush, 2011).

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Morpho-physiological and yield characteristics of interspecific hybrids between cultivated eggplant (*Solanum melongena* L.) and wild relatives in response to drought stress

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ABSTRACT

Drought has been identified as one of the principal global problems, which further exacerbates under climate change. Wild relatives of crops are a genetic resource with an array of traits of interest, including tolerance to biotic and abiotic stresses. The aim of this study was to evaluate the drought tolerance ability of ten interspecific hybrids between seven different cultivated varieties of Solanum melongena L. and three wild relatives (Solanum insanum, Solanum incanum and Solanum lichtensteinii). The Experiment was conducted in a protected house at the University Research Station, Meewatrura, Peradeniya (WM2b) during Maha 2018. The experimental materials were subjected to three irrigation treatments viz: field capacity (control, I1) and two droughts stress levels (70% and 40% of field capacity: I2 and I3, respectively). Plant morphological characters, physiological characters and yield attributes were measured. Results revealed that drought stress (I2 and I3 treatments) significantly (pd 0.05) reduced the plant height, canopy width, number of leaves, number of branches, number of fruits and finally the average yield of all interspecific hybrids. The proline content and chlorophyll concentrations (a, b and total) were significantly increased (pd 0.05) in the plants under the drought stress. Relative water content also significantly increased (pd 0.05) for I3 level. Total soluble solids of fruits were increased significantly (pd 0.05) due to the drought stress. Moreover, interspecific hybrids MEL2 × S. insanum, MEL3 × S. insanum, MEL5 × S. insanum, MEL6 × S. insanum and MEL7 × S. insanum, have shown better performance under I1 and I2 treatments while, MEL2 × S. insanum, MEL3 × S. insanum, MEL5 × S. insanum, MEL6 × S. insanum, MEL7 × S. insanum showed best tolerance under I3 treatment. Thus, those interspecific hybrids have potential to utilize as genetic materials for future breeding programs to develop drought resistant eggplant varieties.

Keywords **:** Drought stress, Eggplant, Interspecific hybrids, *Solanum incanum*, *S. insanum*, *S. lichtensteinii*, *S. melongena*

INTRODUCTION

In many regions of the world, there have been considerable changes in the nature of droughts, floods and extreme temperature events since the middle of the twentieth century. Now onwards at least 0.2 °C per decade average increment of temperature is projected (Liu *et al.*, 2019). Annual average rainfall of Sri Lanka has been decreasing for the last 57 years at a rate of about 7 mm per year. Agricultural crops are affected by global warming due to increment of atmospheric CO_2 concentration and changing of climatic conditions (De Costa, 2008). Global warming results in erratic climate change and the reduced groundwater availability because of competition the use of ground water for industrial needs (Green *et al*., 2011). Increase the crop production selection of new genotypes having resistant to abiotic stresses such as water deficit, salinity, extreme temperatures is essential (Ashraf *et al.*, 2009). Domestication process cause to severe reduction in genetic diversity of most crops when comparing with their wild relatives (Smýka *et al*., 2018). Wild relatives of crops inherent largely untapped genetic diversity (Momin *et al*., 2016) for biotic and abiotic stress resistance, and could greatly expand the available domesticated gene pools to assist crops to survive in the predicted extremes of climate change. Genomic strategies can obtain in the introgression of these valuable characteristics into the domesticated crop gene pools, it is key issue for evaluated for crop improvement (Zhang *et al.*, 2017). Eggplant takes over a half an year developing time under warm climatic conditions

to give preferred high quality fruits and it can be classified as a moderately sensitive vegetable crop for drought (Ghaemi and Rafiee, 2016). To develop crops having tolerance to drought it is necessary to identify genetic variability for drought among crop varieties, or among sexually compatible species to incorporate drought-tolerance together with appropriate agronomic traits. The morpho-physiological changes in response to drought stress can be used to identify tolerant genotypes to develop new varieties with better productivity under drought stress (Nadeem *et al*., 2019). Plant height, canopy width, number of leaves leaf area, dry biomass are reliable morphological data to evaluate the response of plants to drought stress. While stomatal conductance, transpiration rate, photosynthesis rate, proline content and water use efficiency are physiological plants responses (Anjum *et al.*, 2017).

MATERIALS AND METHODS

Accessions and experimental site

The experiment was carried out in a green house at the University Research Station, located in Meewathura, Faculty of Agriculture, University of Peradeniya, during June - December 2018 (*Maha)* under controlled conditions where maximum and minimum temperatures were around 42.7 °C and 17.4 °C, respectively. Ten interspecific hybrids were used which were developed using six cultivated eggplant varieties and three wild relatives as parents with diverse origin (Table 1).

Table 1. Details of the plant materials used in the study

Cultivated Eggplant accessions (*S. melongena* L.)	**Origin**	**Wild relatives**	**Origin**	**Interspecific hybrids**
MEL 2	Ivory Coast	*S. insanum* (INS 1)	Sri Lanka	MEL 2 × INS 1
MEL 3				MEL 3 × INS 1
MEL 4	Sri Lanka	*S. lichtensteinii* (LIC 1)	South Africa	MEL 4 × INS 1 MEL 4 × INS 1 MEL 4 × INC 1 MEL 4 × LIC 1
MEL 5				MEL 5 × INS 1 MEL 5 × INC 1
MEL 6		*S. incanum* (INC 1)	Israel	MEL 6 × INS 1
MEL 7				MEL 7 × INS 1

Experimental design

A completely randomized design was used which consists of 10 interspecific hybrids and three levels of irrigation treatments with three replications. Polybags (25 cm ´ 35 cm) filled with a mixture of top soil : compost : coir dust: half burn paddy husk at the ratio of 5: 3: 2: 1. The optimum water quantity was determined according to Eunice (2014). Irrigation treatments were applied based on the filed capacity (FC) of potting media. Three drought stress levels were adopted based on field capacity namely; I1; FC/ Optimum watering (3.5 L), I2: 70% FC (2.5 L) and I3: 40% FC (1.5 L).

Data Collection

Physiological data and relative water content (RWC) of leaves were measured using the third leaf of main stem of each plant (Matricaria, 2011), Leaf Chlorophyll was measured according to Pirzad *et al*. (2011) and Proline was measured using colorimetric method used by Bates *et al*. (1973). Plant height (cm), plant canopy width (cm), stem diameter (cm), number of green leaves per plant (LPP), number of branches, leaf area were measured as morphological data and yield per plant (YPP/g), Number of fruits per plant (NOF), Mean fruit weight (MFW/g) total soluble solids (TSS) were also measured.

Data analysis

Analysis of variance (ANOVA) was performed using SAS statistical program (SAS 9.1.3 version) at 5% level of significance. Mean separation was done using Duncan Multiple Range test (DMRT).

RESULTS AND DISCUSSION

A highly significant difference was observed between irrigation levels and accessions for all measured traits. Mean performance of all morphological traits including plant height, canopy width, LPP (Fig. 1) and number of branches decreased significantly (at $p<0.05$) from I1 to I3. Drought had the most significant effect on growth traits at I3 and less effect was observed at I2. Drought has been widely reported to hinder growth (Mofokeng and Mokgehle, 2019; Hafeez *et al*., 2015). The reduction in the LPP and branches under drought stress is another mechanism that plants use to reduce the surface area available for transpiration. This helps to increase water use efficiency in metabolic processes in plant (Pucholt *et al.*, 2015). Proline concentration and Chlorophyll (a, b and total) concentration of leaves was highly significant between accessions and drought stress levels ($p<0.05$) (Fig 2, 3 and 4). Results of the present study is in agreement with Chartzoulakis and Noitsakis, (1993) and Mensah *et al*. (2006). Level of proline concentration was significantly higher in I3 compared to I1 and I2 levels. Accumulation of proline has been reported under the drought stress in eggplant (*Solanum melonogena*) (Laxman *et al.*, 2011). Under water deficit conditions the decline in osmotic potential achieved by solute accumulation such as proline (Heuer, 2010).

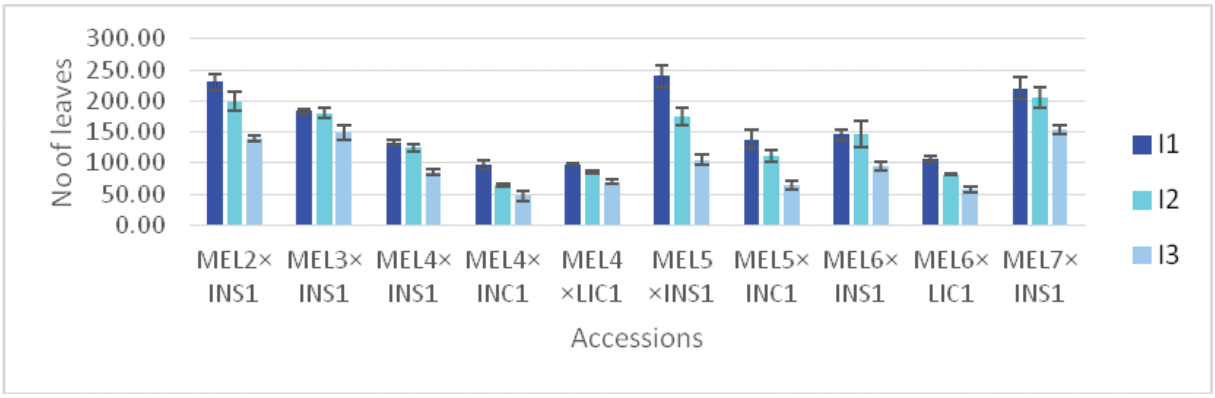

Figure 1. Number of leaves variation under different irrigation treatments

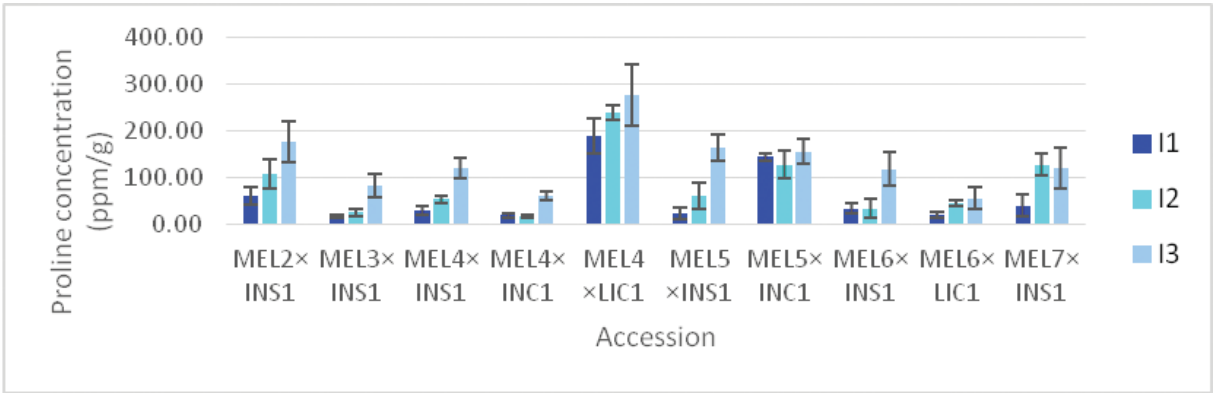

Figure 2. Leaf proline concentration variation under irrigation treatments

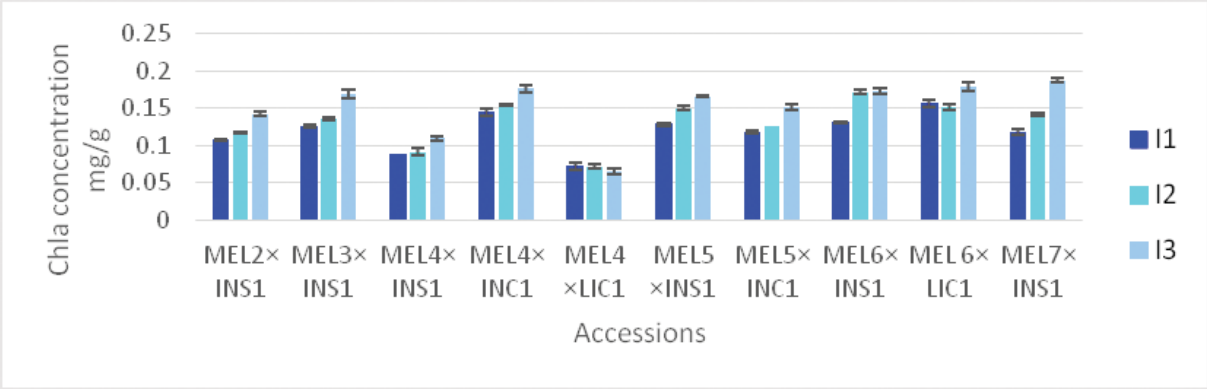

Figure 3. Leaf chlorophyll (a) a concentration variation under different irrigation treatments

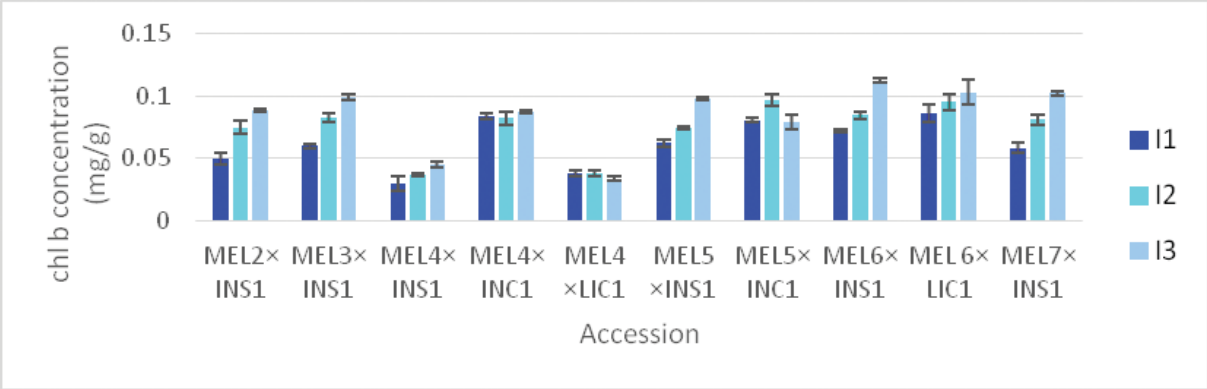

Figure 4. Leaf chlorophyll (b) concentration variation under different irrigation treatments

RWC was significantly different between drought stress levels as well as between accessions (Fig. 5). The high water content maintained by plants under drought stress had produced higher yield (Pirzad *et al.*, 2011).

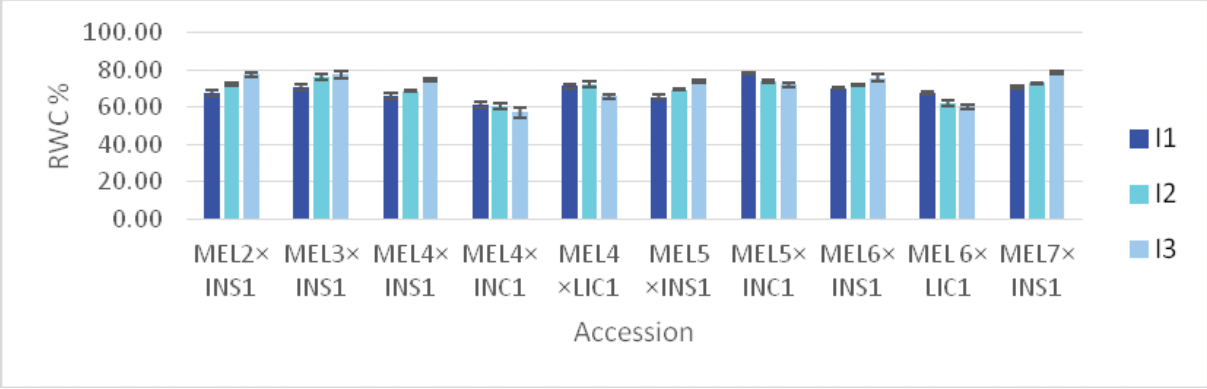

Figure 5. Leaf relative water content variation under different irrigation treatments

YPP (Fig. 6) and NOF (Fig. 7) were significantly reduced from I1 to I3. Moreover, TSS (Fig. 8) was significantly increased ($p<0.05$) as drought stress increased. However, MFW was not affected by the drought stress levels (Fig.9). Drought during vegetative phase affects plant's assimilatory organs, which usually leads to decrease in number and size of the fruits resulting in lower photosynthetic production (Chaves *et al*., 2003). As a result, yield decreases due to less amount of assimilate available for the developing fruits. Duration of drought stress affect the number of flowers leading to a decrease in the number of fruits and the marketable yield (Bidel, 2014) and premature flower drop (Southwick and Davenport, 1986). According to (Mustapha *et al*., 2014) drought stressed plants produce chemical substances such as amino acids which lead to increase soluble solids in fruits.

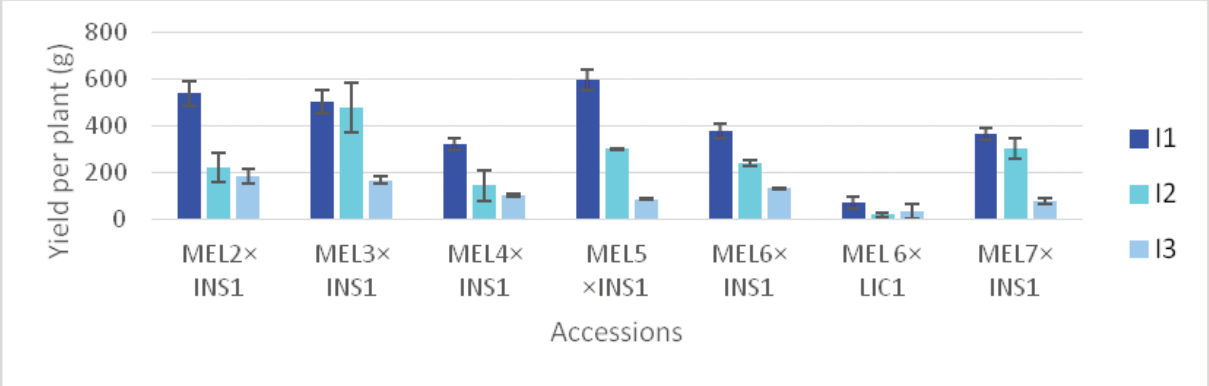

Figure 6. YPP variation under different irrigation treatments

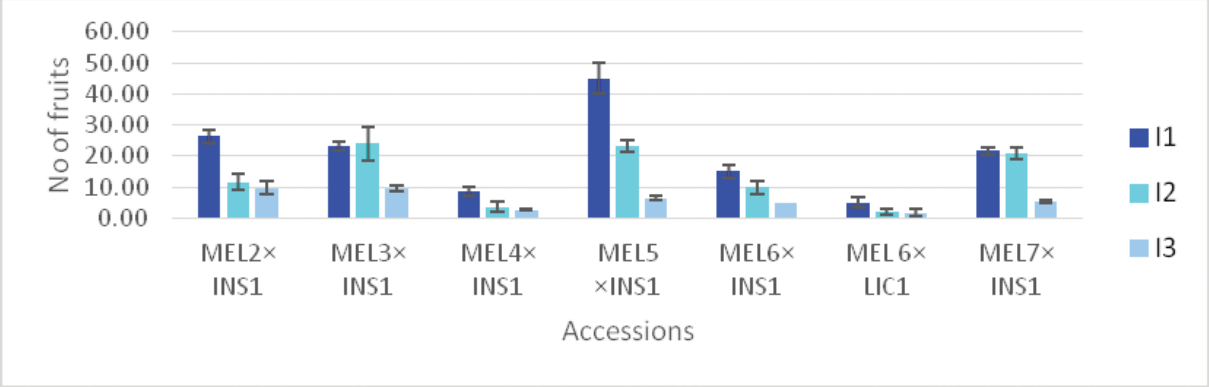

Figure 7. NOF variation under different irrigation treatments

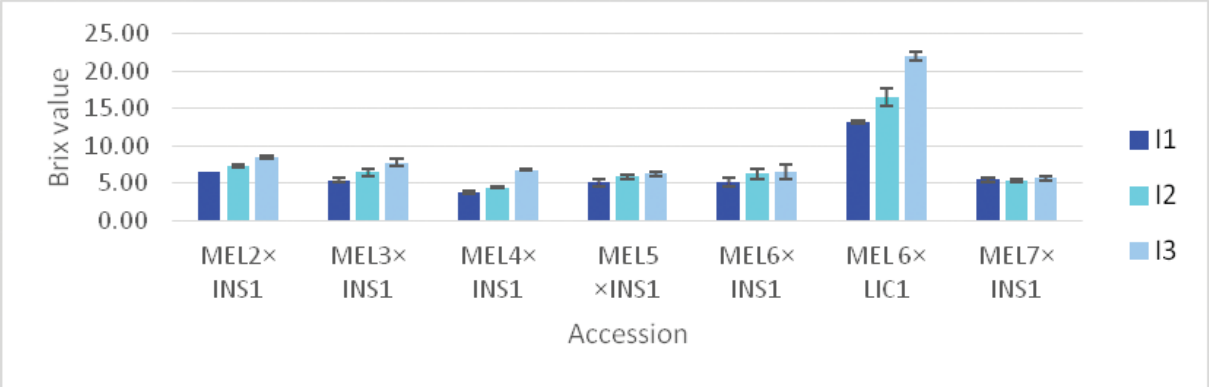

Figure 8. TSS of fruits variation under different irrigation treatments

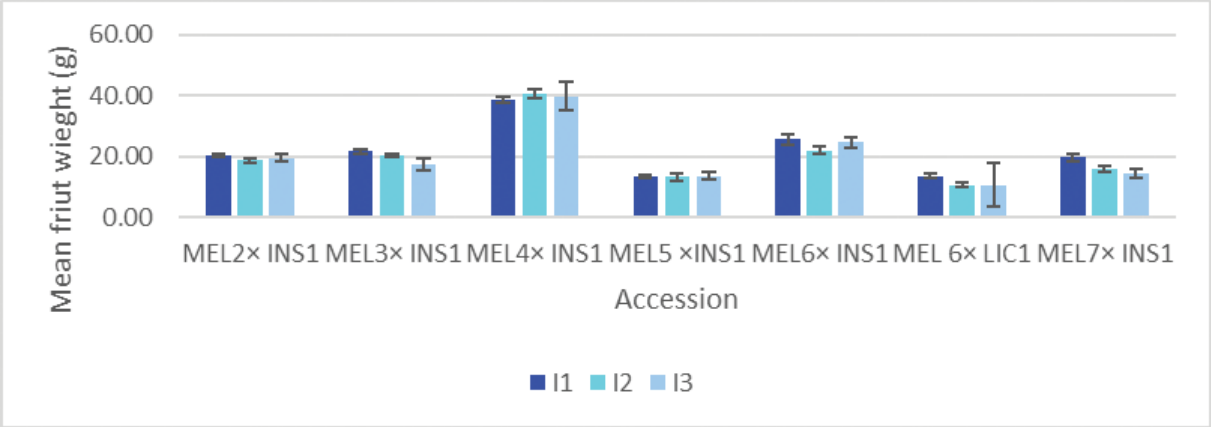

Figure 9. FW variation under different irrigation treatments

The data were analyzed using principal component method. The scree plot shows that the first four factors account for most of the total variability in data. The remaining factors account for a very small proportion of the variability and are likely unimportant (Fig. 10).

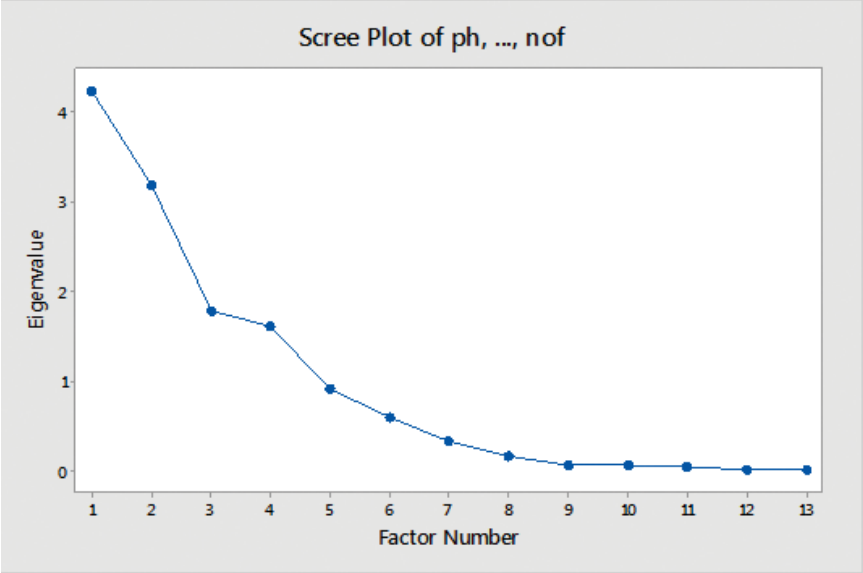

Figure 10. Scree plot under factor analysis where factor 1: LPP, number of branches per plant, plant canopy width, YPP and NOF, factor 2: Chlorophyll concentration (a, b and total), TSS value of fruits and MFW, factor 3: RWC and proline concentration and factor 4: Plant height.

CONCLUSION

Interspecific hybrids MEL2 × *S. insanum*, MEL5 × *S. insanum*, MEL7 × *S. insanum*, MEL7× *S. insanum* and MEL6 × *S. insanum* showed promising morphological and yield characteristics under I1 and I2. Interspecific hybrids MEL3 × *S. insanum*, MEL2 × *S. insanum*, MEL7 × *S. insanum* and MEL6 × *S. insanum* and MEL5 × *S. insanum* showed the highest morphological, physiological and yield characters under I3. Interspecific hybrid MEL3 × *S. insanum*, MEL2 × *S. insanum*, MEL4 × *S. insanum*, MEL6 × *S. insanum* and MEL5 × *S. insanum* have potential to utilize as genetic material for future breeding program related to drought tolerance or avoidance.

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Evaluation of fruit characteristics of *Elaeagnus latifolia* L. in the north eastern hill region, India

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ABSTRACT

*Elaeagnus latifolia L., locally known as Sohshang is a very important fruit species among the tribes of Meghalaya. The fruit has been grown for its edible fruits and ornamental value since time immemorial. A study was carried out to find the variation among genotypes of the species during 2015-17. Result showed significant variation among genotypes ($p \leq 0.05$). RCE-2 was found to produce highest fruit length (3.63 cm) and fruit diameter (2.84 cm), while, maximum fruit weight and edible flesh content was observed in RCE-2 (21.79 g) and RCE-1 (81.09%), respectively. RCE-2 produced maximum value for all seed characteristics. Total soluble solid was recorded maximum in RCE-4 (11.2%), titratable acidity in RCE-2 (4.03%), fruit juice pH in RCE-4 (3.7) and ascorbic acid in RCE-3 (15.03 mg 100 g^{-1} pulp). Similarly, a significant correlation was obtained among different physico-chemical traits. Fruit weight showed positive correlation with edible flesh (0.856**), seed weight (0.9210**), titratable acidity (0.867**), but negatively correlated with total soluble solid (-0.774*). Edible flesh had positive correlation with titratable acidity (0.903**) while had negative correlation with TSS (-0.878**) and ascorbic acid (-0.707*). Therefore, in view of the above, popularization of the crops is the need of the hour. Variation observed might be useful for selection of promising genotypes and inclusion as parental line in breeding programme.*

Keywords : *Elaeagnus latifolia*, fruit, variation, biochemical

INTRODUCTION

Sohshang (*Eleaegnus latifolia* L.) has traditionally been known for centuries as one of the most potential underutilized fruit crops among the tribal habitat of North Eastern Himalayan region, India (Rymbai *et al*., 2016a). The crop is a member of the family Elaeagnaceae, genus *Elaeagnus* which is vernacularly known as Sohshang in Khasi Hills, Slangi in Jaintia Hills and Muslerhi in Sikkim. Geographically, the region expanses between 21°50¹ and 29°34¹ N latitude and 85°34¹ and 97°50¹ E longitude, with elevation varies from near sea-level to over 7,000 m above MSL. The shrub occurs very commonly in the foothills of Eastern Himalayas, which could be observed in large number in the hills of Khasi and Jaintia, Meghalaya, India. It is found to be grown in semi-wild condition in the kitchen garden / or back yard for its ornamental values and edible fruits. It is a perennial and semi-deciduous mutli-stem shrub, belonging to the family Elaeagnaceae. The family consists of three genera, *viz*., *Elaeagnus*, *Hippophae* and *Shepherdia*. The genus *Elaeagnus* consists about 40 species of shrubs and trees, however, only 3 species are known for planting in other part of the world, *viz*., Russian olive (*E. angustifolia*), silverberry (*E. commutate* Bernh. Ex Rydb) and autumn olive (*E. umbellate* Thunb). Apart from fruits, seeds of most of the species including *E. latifolia* are edible. Recently, the genus has become a critical underutilized fruit crops because the trees of the genus *Elaeagnus* have a symbiotic relationship with certain soil bacteria like the genus *Actinomycetes* responsible for producing root nodules and fix atmospheric nitrogen (Follstad Shah *et al*., 2010). Because of its atmospheric nitrogen fixing abilities, an increase in fruit production up to 10% on intercropping with plum and nuts was reported (Plant for a Future, 2014). It was also observed that the species are quite resistant to high wind velocity and performed well even on nutrient poor acidic soil and soil moisture stress conditions (Rymbai *et al*., 2017). More importantly, the fruits are also capable of minimizing the incidence of cancer and halting or reversing the growth of cancers (Matthews, 1994).

Few researches has been undergoing in this crop, flower morphology of *E. latifolia* has already

been reported by Rymbai *et al*. (2017), which noted a hermaphrodite and actinomorphic flower promote selfing and outcrossing. In addition, reports of identity of hyperparasite *Simplicillium lanosoniveum*on *Aecidium elaeagni-latifoliae* in Umiam (Baiswar *et al*., 2014), standardization of agro-techniques and strategies for development of the crop (Deka and Rymbai, 2014) has also been made an efforts. However, information on fruit and biochemical characteristics of this fruirs are sporadically available. Therefore, attempt is made to study the physico-chemical characteristics of *E. latifolia* for better understanding of its fruit characteristics and its potential utilization.

MATERIAL AND METHODS

Experiment was carried out during 2015-17 in five genotypes of *Elaeagnus latifolia* planted in the Horticulture Experimental Farm of the Division of Horticulture, ICAR Research Complex for NEH Region, Umiam, Meghalaya. About 20 twenty matured fruits were collected randomly from all directions of the tree for analysis of variability exist among genotypes with respect to physical and biochemical characteristics. Fruit samples were washed and kept at room temperature for 10 minutes to remove the adhering water before analysis. The fruit and seed weights were determined using electronic balance (Adiar Dutt-1620C). Fruit length and diameter, seed length and seed diameter were measured using digital verniercallper (Mitutoya Digimatic Caaliper, Code No. 500-147).The edible flesh percentage was calculated as fruit weight – seed weight/ fruit weight x 100. The total soluble solids (TSS) was determined with the help of digital hand refractometer (Model - *HI 96801*) from three different points, *i.e*. shoulder, middle and distal end portion of the fruit after mixing thoroughly. The values were expressed in percentage (Ranganna, 1997). Titratable acidity and ascorbic acid were also estimated as per methods described by Ranganna (1997). The data on different parameters were analyzed using analysis of variance (ANOVA) by employing SPSS (version 14.00). Difference were considered statistically significant at $P \leq 0.05$. Relationship among different parameters were analysed using Pearson's correlation.

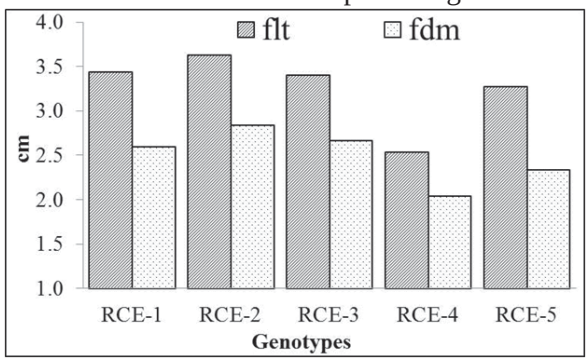

Fig. 1. Fruit length (flt) and fruit diameter (fdm) of *Elaeagnus latifolia*

RESULT AND DISCUSSION

Physical characteristics

Result indicate significant different among genotypes ($p \leq 0.05$). RCE-2 was found to produce highest fruit length (3.63 cm) and fruit diameter (2.84 cm), which was followed by RCE-1 (fruit length, 3.43 cm**)** and RCE -3 (fruit diameter, 2.66 cm; Fig 1). Similarly, maximum fruit weight was noted in RCE-2 (21.79 g) followed by RCE-1 (16.60 g). Edible flesh content was recorded highest in RCE-1 (81.09%), which was followed by RCE-2 (79.30%; Fig 2). Seed characteristics were recorded maximum in RCE-2 for all the characteristics (Fig 3). RCE-2 showed maximum seed length (3.04 cm) and seed diameter (1.47 cm). Seed weight was recorded highest in RCE-2 (4.51 g) which was significantly higher over all other genotypes. Result showed variation among genotypes which was in accordance with the finding of Devachandra *et al*. (2018). The variation might be due to genetically variation of the genotype (Rymbai *et al*., 2016b).

Biochemical characteristics

Biochemical parameters showed significant different among genotypes ($p \leq 0.05$; Fig 4). Total soluble solid was recorded maximum in RCE-4 (11.2%), followed by RCE-5 (9.2%) and minimum in RCE-3 (8.8%). With regards to titratable acidity, RCE-2 showed highest value (4.03%), followed by RCE-1 (3.74%). Fruit juice pH was highest in RCE-4 (3.7) while lowest in RCE-2 (3.1). Ascorbic acid was recorded highest in RCE-3 (15.03mg 100 g^{-1} pulp), followed by RCE-4 (13.27 mg 100 g^{-1} pulp), while lowest was recorded in RCE -1 (9.83 mg 100

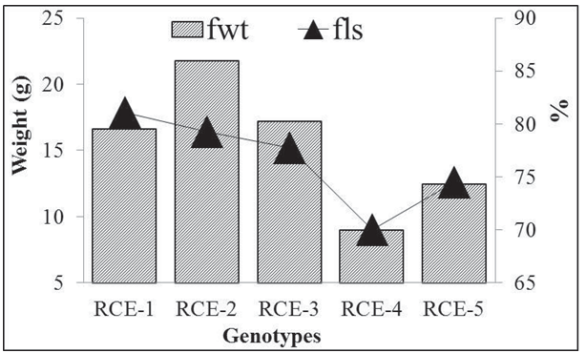

Fig. 2. F**ruit weight (fwt) and edible flesh (fls) of *Elaeagnus latifolia***

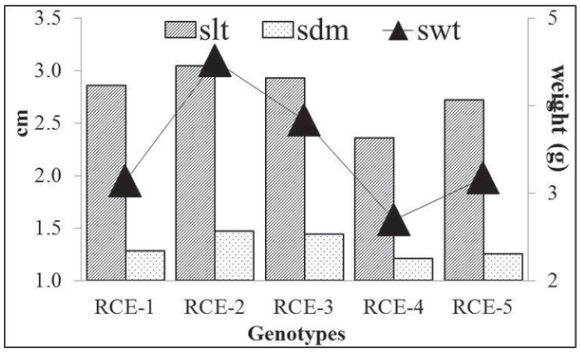

Fig. 3. Seed length (slt), seed diameter (sdm) and seed weight (swt) of *Elaeagnus latifolia*

g^{-1} pulp).Similar trend has also been reported by Hussain (2011) in *E. umbellata*. The variations among genotypes might due to influence of genetical traits of individual genotypes (Rymbai *et al*., 2019).

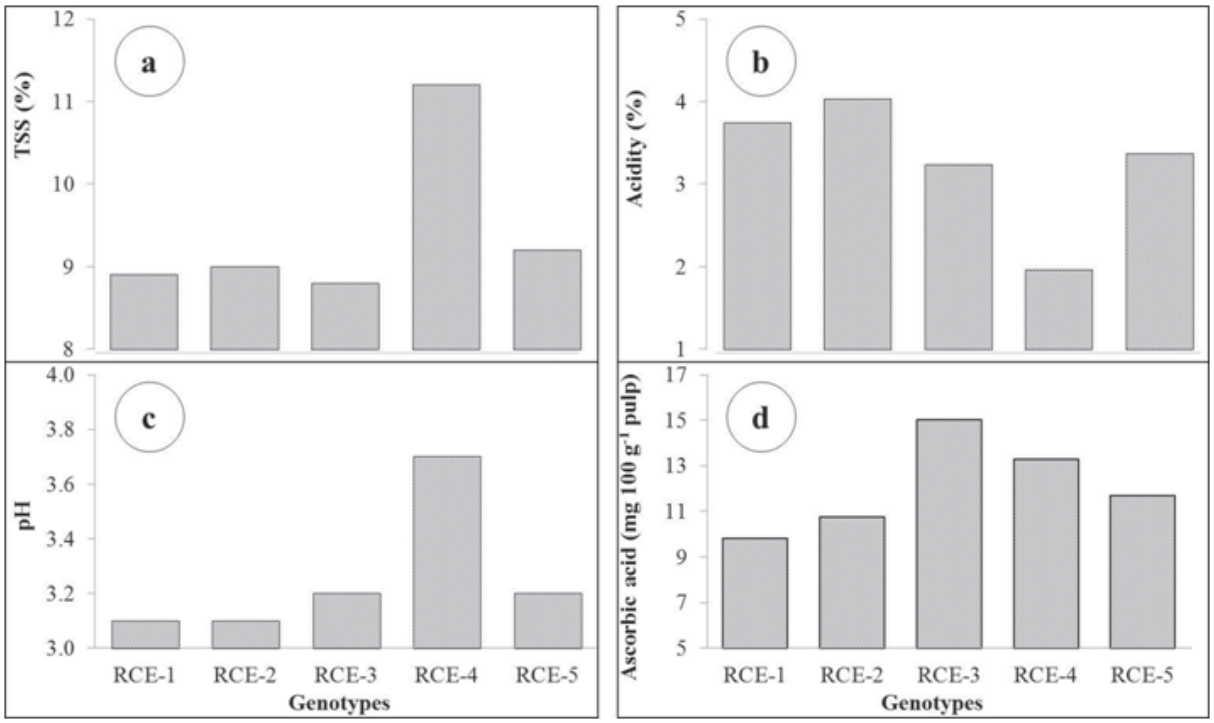

Fig. 4. Biochemical characteristics of *Elaeagnus latifolia*, a – TSS (%), b – Acidity (%), c – pH, d – ascorbic acid (mg 100 g^{-1} pulp)

Relationship among importance characteristics

A significant correlation was observed among different physico-chemical traits of *Elaeagnus latifolia* (Table 1). Fruit weight showed positive correlation with edible flesh (0.856**), seed weight (0.9210**), titratable acidity (0.867**), but negatively correlated with total soluble solid (-0.774*). Edible flesh had positive correlation with titratable acidity (0.903**) while showed negative correlation with TSS (-0.878**) and ascorbic acid (-0.707*). Result indicated that higher the fruit weight tends to produce higher edible parts, while lower might be the total soluble solid. Our finding has also been in line as reported by Bhowmick and Banik (2008).

Table 1. Pearson's correlation coefficient among physico chemical characteristics of *Elaeagnus latifolia*

Characters	Fruit weight	Edible flesh	Seed weight	Total soluble solid	Titratable acidity	Ascorbic acid
Fruit weight	1.000	0.856**	0.920**	-0.774*	0.867**	-0.609
Edible flesh		1.000	0.600	-0.878**	0.903**	-0.507
Seed weight			1.000	-0.632*	0.712*	-0.379
Total soluble solid				1.000	-0.905**	0.495
Titratable acidity					1.000	-0.592
Ascorbic acid						1.000

**significant at P ≤ 0.01; *significant at P ≤ 0.05

CONCLUSION

A significant variation was observed among genotypes of *Elaeagnus latifolia* with respect to fruit and seed characteristics. It divulged that RCE-2 and RCE-3 can be potential genotypes, which allowed for further selection for commercial cultivation. In addition, these genetic resources can be employed in breeding programme.

ACKNOWLEDGEMENT

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Propagation of *Bauhinia kockiana* Korth through stem cuttings as affected by maturity stage of cuttings and different biofertilizers

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ABSTRACT

Bauhinia kockiana Korth (Kock's Bauhinia) belongs to family Fabaceae and is consisting with effective anti-cancer substances. Currently plant is propagated by air layering and it takes longer duration to flower. Also it is not practicable for mass propagation. Thus, the present study was conducted to find out the suitability of stem cuttings as mass propagation method. Three types of stem cuttings (Top, Semi-Hard Wood and Hard Wood) with four types of bio fertilizers (jeewamruthum, vermi wash, fish tonic and indo chinese traditional microbial culture) were used for the experiment. Experiment was arranged as Complete Randomize Design with ten replicates per treatment at Seethawaka wet zone botanic gardens, Avissawella. Bio Charcoal & River Sand in 1:1 ratio was used as potting medium. Growth parameters were collected in weekly up to eleven weeks after planting. Data was analyzed using the Mini Tab 17 statistical package. Result revealed that the top cutting with indo chinese microbial culture significantly increased the plant height, cumulative number of new leaves, cumulative number of new buds and the root volume. Therefore, it can be concluded that top cuttings planted in Bio Char & River sand 1:1 potting media treatd with indo chinese microbial culture could be used for propagation of Bauhinia kockiana Korth .

Keywords : Bauhinia kockiana Korth, Jeewamruthum, Vermiwash, Fish Tonic, Indo Chinese Microbial Culture

INTRODUCTION

Kock's Bauhinia (*Bauhinia kockiana* Korth.) is belongs to family Fabaceae and is a floricultural crop, native to Malaysia and Indonesia, which is mostly using as one of the major landscaping floricultural crops in Sri Lanka for constructing the landscape gardening as an excellent specimen for trellises, arbors, arches, arch ways, pergolas and using for ornamental purposes in home garden exterior decorations as cascading over a garden wall, lamp posts and for fences due to spectacular attractive blooms. *Bauhinia kockiana* Korth. is a perennial semi-deciduous vigorous creeper type woody plant that can reach a height of about 10-15 feet in gardens. The flowers of *B.kockiana* Korthare in large clusters that open yellow but gradually turn to scarlet-orange and blooming throughout the year. Cut flowers can be kept for about 5 days without any colour change on the petals (Chong *et al*., 2009).

This plant also has medicinal value and used several parts to treat various health complications. For instance, its roots are used by the Kelabit ethnic group in Sarawak, eastern Malaysia to treat gonorrhoea, nervous debility, insomnia and fatigue. The infusion of bark and root are also used traditionally to treat toothache. *B. kockiana* flowers enrich with anticancer properties and anticancer agents. So the flowers can be used to prevent and treat for the cancers. A study reported that *B. kockiana* plant exhibited fairly strong antioxidant and antimicrobial activities. Some papers had focused and founded on the assessment of antibacterial activity of *B. kockiana* towards methicillin-resistance *Staphylococcus aureus* (MRSA), to purify and to identify the antibacterial compounds, and to determine the mechanism of antibacterial activity (Chew *et al*., 2014).

Since, the huge demand for *B. kockiana* Korth in the local market among the landscape designers, the supply is not matching by the growers. This plant is currently propagated through layering only as done in many fruit plants like pomegranate (Bhagwa *et al*., 2017) but most of the local growers are now discouraging to plant production of *B. kockiana* Korth due to the higher time consuming and less number of plants are produced from layering. Therefore, main objective of this study

was to evaluate the most suitable maturity stage of the cuttings of *B. kockiana* Korth on rooting as affected by different bio fertilizers that can be prepared in locally and easily available for the growers.

MATERIALS AND METHOD

Stem cuttings of *Bauhinia kockiana* Korth were collected from a vigorous mother plants maintained at the Seethawaka Wet zone Botanic Gardens-Avissawella, Sri Lanka. There were three types of stem cuttings (top cutting, semi-hard wood cutting & hard wood cutting). All the cuttings were in same length with four leaves, that were removed half of the leaf blade and having 3-4 active buds. Before entering the stem cuttings in to the potting medium of Bio Char and River Sand in 1:1 ratio, treated with 3 ml each of different bio fertilizers viz., Jeewamruthum, Fish Tonic, Vermy Wash and Indo Chinese Traditional Microbial Culture.

The Jeewamruthum, Fish Tonic, Vermy Wash were in liquid phrase and the Indo Chinese Microbial Culture was in semi-solid phrase. So the all three types of the stem cutting were planted in the inert medium as about 3cm inside the inert medium to maintain a constant height. All the stem cuttings were in same height (15cm). Before planting the stem cuttings, the each and every plating pot with the potting medium had treated with 3ml from Jeewamruthum, Fish Tonic and Vermy Wash by drech them into the potting medium and mixed well the pottiug medium. We used 3mg of Indo Chinese Microbial Culture and mixed through the pulp in the potting medium. Then pots were irrigated properly and carefully. After the irrigation, the planting pots were introduced to Plant Propagator. After placing all the planting pots in the inside of plant propagator it was closed for the better maintained of temperature and the relative humidity inside the Plant Propagator. After one week from the planting the pots were treated again according to the treatment schedule. It was used 3ml from Jeewamruthum, Fish Tonic and Vermy Wash for the in-cooperation to the medium without disturbing to the stem cutting. 3g of the Indo-Chinese Traditional Culture was mixed with 3ml of de-choronized water was used and in-cooperate to the medium. The same was applied for the two weeks and three weeks after planting the stem cuttings.

Treatment scheduled was as T 1- Top Cutting with Jeewamruthum; T2- Top Cutting with Vermi Wash; T3- Top Cutting with Fish Tonic; T4- Top Cutting with Indo- Chinese Traditional Microbial Culture; T5- Semi-Hard Wood Cutting with Jeewamruthum; T 6- Semi-Hard Wood Cutting with Vermi Wash; T 7- Semi-Hard Wood Cutting with Fish Tonic; T 8- Semi-Hard Wood Cutting with Indo- Chinese Traditional Microbial Culture; T 9- Hard Wood Cutting with Jeewamruthum; T 10- Hard Wood Cutting with Vermi Wash; T 11- Hard Wood Cutting with Fish Tonic; T 12- Hard Wood Cutting with Indo- Chinese Traditional Microbial Culture; T 13- Top Cutting with Rooting Hormone; T 14- Semi-Hard Wood Cutting with Rooting Hormone (Control) and T 15- Hard Wood Cutting with Rooting Hormone. So, total number of treatment combination=15; Number of replication (cuttings taken) in each treatment=10 and thereby total number of cuttings used in the experiment=150. The experimental design was two factor Complete Randamozised Design. The cuttings were tested separately in each week up to 12 weeks period inside the plant propagator.

Just after establishment of plants in pots, water well before introducing the bio fertilizers kept in the plant propagator. Data was collected in each weekly up to 11 weeks. Cumulative plant height, cumulative number of new leaves per cutting, cumulative number of new buds per cutting, root length and the root volume were measured as growth parameters. Statistical analysis was performed by using ANOVA in Mini Tab 17. Grouping was done to determine the significance among clusters.

RESULTS AND DISCUSSION

Results revealed that, the significantly highest cumulative plant height was recorded in top cuttings treated with indo Chinese traditional microbial culture, followed by top cuttings with jeewamurthum medium and semi hardwood cutting treated with Indo Chinese traditional microbial culture at 2 weeks after planting (Figure 1). The same result was recorded in 5 weeks after planting also (Figure 2) and 11 weeks after planting (Figure 3).

Result revealed that the excretions secreted by the microbes can induce the plant growth. The results had identified by Rini *et al* (2014) on *Piper nigrum* L. it had indicated that the maximun increase in the plant height had recorded by the bio fertilizers used for the experiment. Similar observations were reported by Kiran *et al.* (2012). According to the findings of Devakumar *et al*. (2014) that higher number of bacteria, different fungi and N-fixers clearly indicate that the jeevamruthum is enriched consortia of native soil microorganisms. Due to the higher beneficial microbial load would mobilize more of plant nutrients and provide plant growth promoting substances and also other micro nutrients required by the plants. Result of the research on gherkin cultivation of Devapriya and Yapa (2017) again proved the result that newly introduced bio fertilizers- earthworm cast treated with Jeewamruthum + compost, Indonesian bio-fertilizer are the most suitable fertilizer category.

Number of New Leaves per Cutting

The significantly highest cumulative number of leaves was recorded in top cutting with indo chinese traditional microbial culture and semi hardwood cutting with indo chinese traditional microbial culture among the all treatments in 6 weeks after planting (Figure 4). Top cutting with indo chinese traditional microbial culture, top cuttings with jeewamurthum medium and semi hardwood cutting with indo chinese traditional microbial culture were significantly effect for the cumulative number of leaves in 11 weeks after planting (Figure 5).

Due to the substances that is secreting by the microbes, it is inducing the growth of leaves of the *Bauhinia kockiana*stem cuttings. The most significant growth of leaves or the cumulative number of new leaves was recorded by the top cutting with the indo chinese traditional microbial culture. Bio fertilizers were found very effective on the plant growth especially on healthy leaf production. Sadanshu *et al* (2009) reported that bio fertilizers are considered to be a panacea for the prosperity of agriculture. The effect of bio fertilizers on the growth improvement was suggested by Muhammed (2010).

Number of New Buds per Cutting

Results emphasized that, the significantly highest cumulative number of the new buds was recorded in top cutting with indo chinese traditional microbial culture, top cuttings with jeewamurthum medium and semi hardwood cutting with indo chinese traditional microbial culture among the all treatments in 5 weeks after planting (Figure 6). Although in the 11 weeks after planting it had recorded that was significantly highest cumulative number of the new buds in top cutting with indo chinese traditional microbial culture, top cuttings with jeewamurthum medium, semi hardwood cutting with indo chinese traditional microbial culture and in the hard wood cutting with indo Chinese microbial culture among the all treatments(Figure 7).

Sladky and Tichy (1959) compared the effects of foliar or nutrient solution application of the humic substances on shoots. Young leaves responded to a greater extent than older ones. Previous studies reported that bio fertilizers had improved soil productivity, fertility and the propagation, which improved the yield and quality in the floricultural crops. (Dinesh *et al*., 2010) Application of foliar bio fertilizer spray on begonia plants (Sladky, 1959) yielded similar results had recorded.

Root Length

The significantly highest length of the roots was recorded in top cutting with indo chinese traditional microbial culture, top cuttings with jeewamurthum medium, hardwood cutting with indo chinese traditional microbial culture, top cutting with rooting hormone, semi hard wood cutting with rooting hormone and hard wood cutting with rooting hormone among the all treatments in 11 weeks after planting (Figure 8).

Similar results had overview by Ramya (2014) in the *Piper nigrum* L. cuttings that had used to propagate by using the bio fertilizers. By using the chemically synthesized rooting hormone can react with the plant physiology in various manners and can induce the root growth than other solution which had used in this experiment.

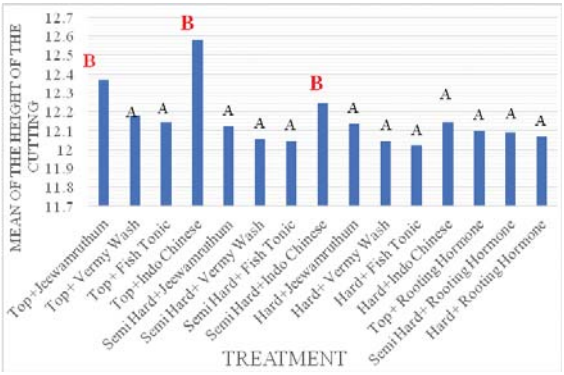

Figure 1: Effect of cutting type and different bio-fertilizers on mean shoot height of *Bauhinia kockiana*. Korth, 2 weeks after planting.
Means on the bars represent the same letter are not significantly different at P d™ 0.05 probability level.

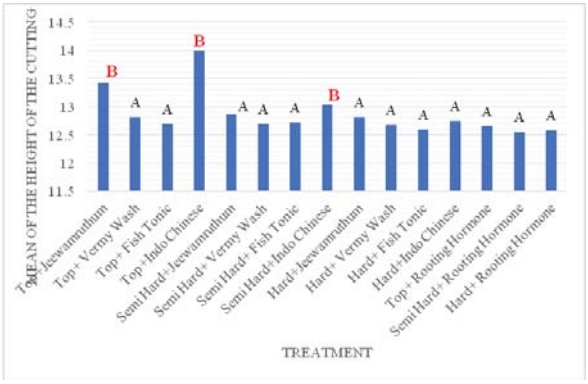

Figure 2: Effect of cutting type and different bio-fertilizers on mean shoot height of *Bauhinia kockiana*. Korth, 5 weeks after planting.
Means on the bars represent the same letter are not significantly different at P d" 0.05 probability level.

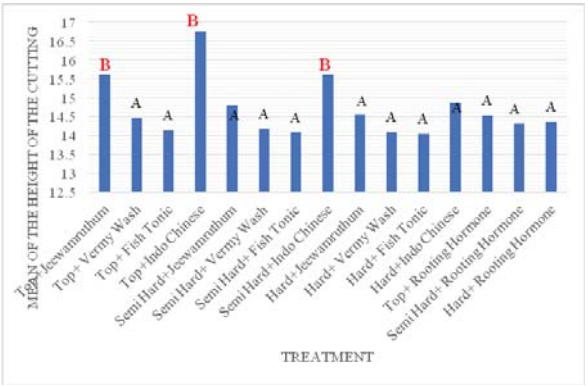

Figure 3: Effect of cutting type and different bio-fertilizers on mean shoot height of *Bauhinia kockiana*. Korth 11 weeks after planting.
Means on the bars represent the same letter are not significantly different at P d" 0.05 probability level.

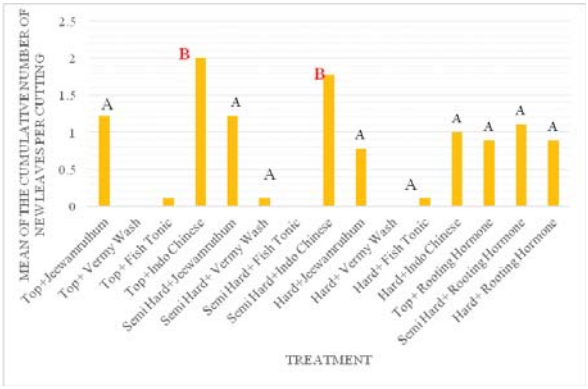

Figure 4: Effect of cutting type and different bio-fertilizers on mean number of leaves in *Bauhinia kockiana* Korth, 6 weeks after planting.
Means on the bars represent the same letter are not significantly different at P d" 0.05 probability level.

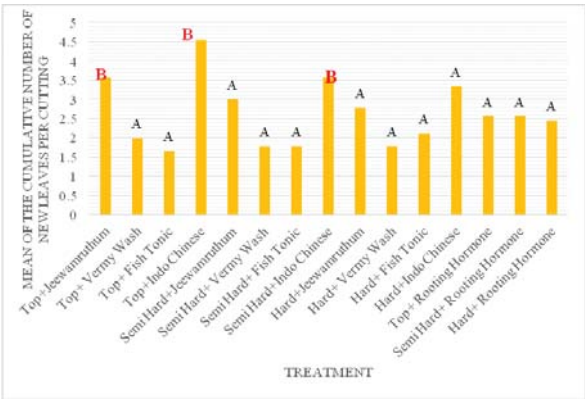

Figure 5: Effect of cutting type and different bio-fertilizers on mean number of leaves in*Bauhinia kockiana* Korth 11 weeks after planting.
Means on the bars represent the same letter are not significantly different at P de 0.05 probability level.

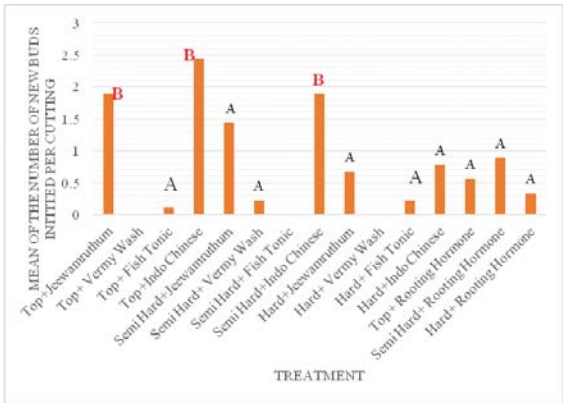

Figure 6: Effect of cutting type and different bio-fertilizers on mean number of new buds in *Bauhinia kockiana* Korth 5 weeks after planting.
Means on the bars represent the same letter are not significantly different at P de 0.05 probability level.

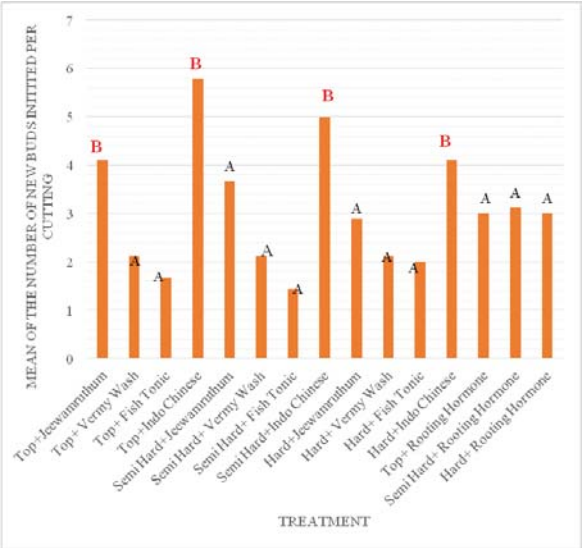

Figure 7: Effect of cutting type and different bio-fertilizers on mean number of new buds in *Bauhinia kockiana* Korth 11 weeks after planting.
Means on the bars represent the same letter are not significantly different at P d" 0.05 probability level.

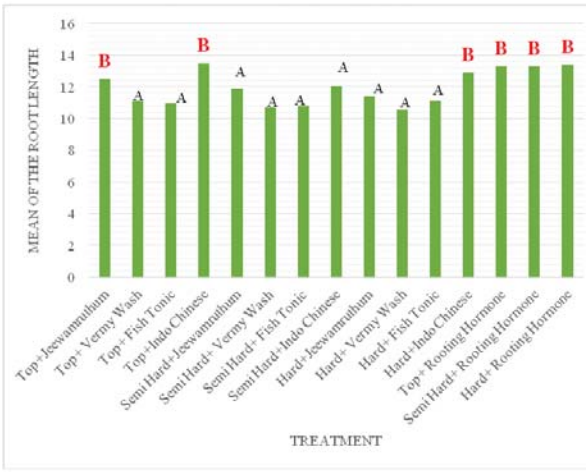

Figure 8: Effect of cutting type and different bio-fertilizers on mean root length in *Bauhinia kockiana* Korth 11 weeks after planting.
Means on the bars represent the same letter are not significantly different at P d" 0.05 probability level.

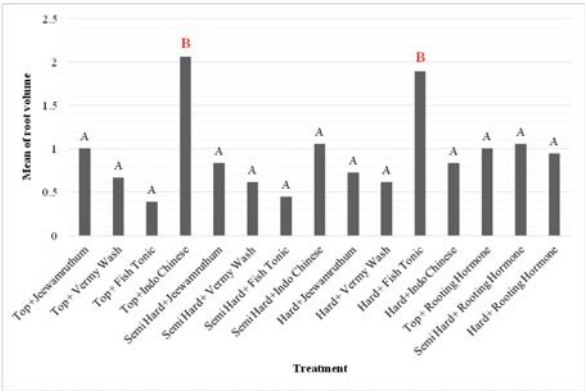

Figure 9: Effect of cutting type and different bio-fertilizers on mean root volume in *Bauhinia kockiana* Korth 11 weeks after planting.
Means on the bars represent the same letter are not significantly different at P d" 0.05 probability level.

Root Volume

Results had shown that, top cutting with indo chinese traditional microbial culture and hard wood cutting with the fish tonic were significantly affect for the amount of the root volume among the all treatments in 11 weeks after planting (Figure 9).

Most of the reports on the usage of bio fertilizers are emphasizing the efficacy of bio control agents in enhancing the plant growth, root growth and the root volume in addition to their ability in increasing the yield. The results of the present study are in agreement with Manoranjitham *et al*. (2000) and Manomohandas (2001). Plant growth regulators like gibberllins, cytokinins and indole acetic acid (IAA) induced by the strains might have contributed for better plant growth and development (Dubeikovsky *et al*., 1993).

The growth observations like plant height and number of leaves were also maximum in the treatment T4 when compared to the other treatment combinations. This might be due to the cumulative effect of all organic bio fertilizers such as jeewamruthum, vermy wash, fish tonic and indo Chinese traditional microbial culture, due to the good water holding capacity, high porosity, increased surface area that provides many microsites for microbial activity and strong retention of nutrients. Previous studies reported that organic bio fertilizers improved soil productivity and fertility, which improved the propagation (Hossain and Ishimine, 2007, Velamurugan *et al*, 2007, Mohaopatra and Das, 2009 and Dinesh *et al*, 2010).

CONCLUSION

With the using of *Bauhinia kockiana* Korth stem cuttings in this experiment it had resulted that they can propagate through stem cuttings easily rather than using of air layering or marcotting in the propagation for the mass production in the commercial purposes in the floricultural industry in Sri Lanka. Results had highlighted that the type of stem cuttings for the using as a propagation of *Bauhinia kockiana* Korth is the top cutting and the best type of bio fertilizer that can be used to the propagation of *Bauhinia kockiana* Korth is Indo Chinese Traditional Microbial Culture.

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SHORT COMMUNICATION

Effect of gamma radiation on survival rate of *Allamanda cathartica* – An indigenous medicinal plant

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ABSTRACT

Changes in the genetic structure of the plant can produce physiologically and chemically efficient plant types with increased production of secondary metabolites. Mutation is a sudden change in the gene which leads to genetic variations. Gamma radiation is a mutagenic agent used extensively to create variations. An experiment was conducted at the University of Colombo Institute for Agro Technology and Rural Sciences, Weligatta with the objective of determining the effective dose of gamma radiation to induce mutations on *A. cathartica*. Rooted plants were exposed to gamma radiation using "Gamma chamber 1200 Cobalt-60" research irradiator and these treatments were carried out at Horticultural Crop Research and Developmental Institute, Gannoruwa. Treatments applied were 0Gy (control), 30Gy, 60Gy, 90Gy, 120Gy and 150Gy. Treated plants were arranged under shade house condition in Complete Randomized Design with three replications and each replication contained eight plants. Survival rate of the plants were recorded continuously up to four weeks at five days intervals. The mutagenic treatments were tested for lethal dose of 50% and the dose at which 50% of the survival at one month was considered as LD50 values. Data were analyzed using ANOVA in SAS software and treatment means were compared using DMRT. It was found that there were significant ($p>0.05$) difference among the gamma radiation treatments on survival rate of the *Allamanda cathartica* plants. Highest survival rate was found in control treatment and decreased the survival rate with increasing doses of radiations. *A. cathartica* plants showed 50% survival at 90 Gy. It could be concluded that the radiation below 90 Gy should be imposed to induce mutations in *Allamanda cathartica*.

Keywords : *Allamanda cathartica*, gamma radiation, mutation, survival.

INTRODUCTION

Allamanda is a genus of flowering plants in the family, Apocynaceae is widely distributed and an indigenous plant in Sri Lanka. *Allamanda* species are familiar as ornamental plants cultivated for their large, yellow and pink colorful flowers and make attractive to the environmental beautification (De Souza-Silva and Rapini, 2009) and it is easily propagated using semi-hardwood cuttings (Hartmann *et al.*, 2010). It has already been reported that many ornamental plants have higher therapeutic and medicinal values (Rajvanshi and Dwivedi, 2017). *Allamanda cathartica* commonly called as Rukkathana, has various medicinal and ornamental values. This plant is used in ayurvedic and unani system for the treatment of various illnesses due to its bioactive secondary metabolites. For medicinal purpose, the milky sap of *Allamanda* possesses antibacterial and possible anticancer properties. Besides, the leave, roots and flowers have been used in the preparation of a powerful cathartic that helps in bowel movement. It also has the possibility as anti-dermatophytic agent and has effects on gastrointestinal motility (Kampanilya, 2013). The use of induced mutations has played a key role in the improvement of superior plant varieties (Ahloowalia and Maluszynski, 2001; Maluszynski *et al.*, 2004; Jain, 2005). Gamma rays are known to influence plant growth and development by inducing cytological, genetical, biochemical, physiological and morphogenetic changes in cells and tissues (Jan *et al.*, 2010). Hence, considering this, an experiment was conducted with the objective of increasing genetic variation in *Allamanda cathartica* plants and to assess the efficiency of different mutagenic treatments, since basic information on this aspects are limited. The first step was to estimate the LD50

value of gamma radiation dose for survival of the *Allamanda cathartica*. LD 50 value provides a good test of the sensitivity of the material to the mutagenic treatment.

MATERIALS AND METHODS

This experiment was conducted at the University of Colombo Institute for Agro-technology and Rural Sciences, Weligatta, Hambantota, Sri Lanka. Semi hardwood stem cuttings of *Allamanda cathartica* were collected from the mother plant with two years of age in Weligatta area and used for this study. Stem cuttings were dipped in water contained bucket soon after detaching from the plant to avoid wilting. Cuttings containing three nodes were used for the planting. A slant cut was made at the distal end of the cutting using a sharp blade and the cut surface was dipped in a root hormone "ROOCTA" (a.i.: Indole 3 – Butyric Acid, Distributed by Oasis Marketing (Pvt) Ltd).

Black polyethylene pots with diameter of 2 inches and with height of 2 inches were filled with the media contained sand and coir dust in equal parts of volume. Cuttings were planted in prepared pots and maintained inside a propagator for one month of time. Rooted and sprouted plants were hardened for one week of time under the shade condition by gradually exposing the rooted plants to the sun.

Figure 1 : Semi hardwood stem cutting used for propagation

Figure 2 : Hardened plants for the gamma radiation

Healthy plants were selected and exposed to gamma radiation treatments using Gamma chamber 1200 Cobalt-60 research irradiator and these treatments were carried out at the Horticultural Crop Research and Developmental Institute, Gannoruwa, Sri Lanka. The treatments were 0 Gy (control), 30 Gy, 60 Gy, 90 Gy, 120 Gy and 150 Gy. Treated plants were arranged in Complete Randomized Design in a shade house with three replications and each replication contained eight plants. Those plants were maintained under a shade house condition with optimum management practices such as regular watering, application of fertilizers and pesticides.

Survival rate of the plant was recorded continuously for one month at five days intervals. The mutagenic treatments were tested for lethal dose of 50% and the dose at which 50% of the survival at one month after gamma radiation treatment was considered as LD50 values. Collected data were analyzed using ANOVA in SAS software and treatment means were compared using DMRT.

RESULTS AND DISCUSSION

It was found that there was significant ($p<0.05$) differences between the gamma radiation treatments on survival rate of *Allamanda cathartica* plants (Table 1).

The highest survival rate was observed in the control where the treatment received no any gamma radiation doses. Increasing of gamma radiation reduced the survival rate of plants. Lowest survival rate was observed in the treatment received 150 Gy. In the first few days of the treatments plants died very slowly exhibiting a fairly good survival rate. However, with time the survival rate was decreased drastically. This could be due to the damage in plant tissue and breakdown of meristematic cells with time (Tien *et al*., 2000; Kovacs and Keresztes, 2002).

Figure 3 : Dying plants after the gamma radiation treatment

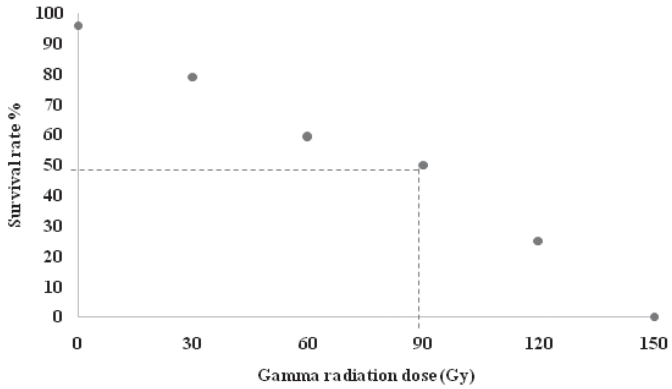

Figure 4 :Effect of gamma radiation on survival rate of *Allamanda cathartica* at 30th day after treatment

Table 1 : Effects of gamma radiation on survival rate (%) of *Allamanda cathartica*

Treatments	5th day	10th day	15th day	20th day	25th day	30th day
T1 (0 Gy)	96^{a}	96^{a}	96^{a}	96^{a}	96^{a}	96^{a}
T2 (30 Gy)	92^{ab}	88^{ab}	79^{b}	79^{a}	79^{b}	79^{b}
T3 (60 Gy)	88^{ab}	79^{b}	71^{b}	59^{b}	59^{c}	59^{c}
T4 (90 Gy)	84^{bc}	75^{b}	67^{b}	54^{bc}	54^{c}	50^{c}
T5 (120 Gy)	75^{cd}	54^{c}	46^{c}	42^{bc}	29^{d}	25^{d}
T6 (150 Gy)	71^{d}	59^{c}	42^{c}	38^{c}	0^{e}	0^{e}
Pr> f	0.0012	0.0002	<0.0001	<0.0001	<0.0001	<0.0001

Means followed by the same superscripts are not significantly different at p>0.05

LD50 value was calculated on the basis of 50 percent reduction of survival rate on 30thday after gamma radiation treatment. The present investigation exhibited that the survival rate of *Allamanda cathartica* decreased with the increase in the dose of the mutagens (Figure 4). About 50% of survival rate at 30th day after treatment was observed in the treatment where 90 Gy of gamma radiation dose was received. These reduction on survival rate of the plant was similar to those of in *Canscoradecurrens*, which is a medicinal plant used in the formulations used to improve intelligence, memory and other higher mental function when different doses of gamma radiations were imposed (Yadav,2016).

Low dose (10-15 Gy) of gamma ray was most positively effective on subsequent growth of plant (Shakhs *et al.,* 2007; Smelkova, 1999) and the radiation is a potential hazard because it can damage DNA and impair physiological processes leading to cytotoxic effects. (Taguchi and Kojima, 2005; Yadav and Kogje, 2015). As indicated by El-Khateeb*et al*. (2016) when *Philodendron scandens* (a plant having ornamental value) were exposed to different doses of gamma radiation decreased the survival rate, which gradually decreased as the gamma dosage increased. The effect of gamma rays on plant survival was gradual depending on the exposure dose, irrespective of the irradiation method (Sawangmee *et al.,* 2011).

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