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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).

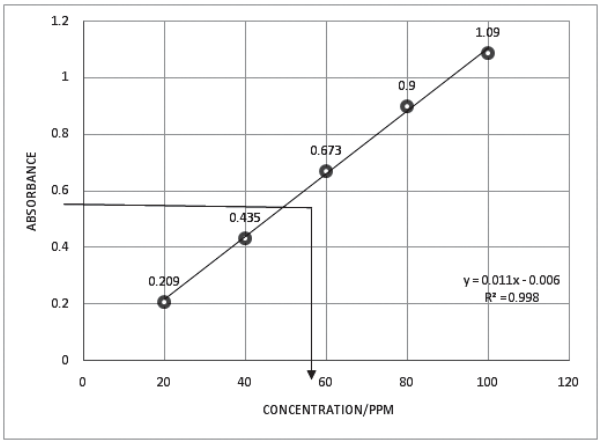

Figure 1. Standard Gallic acid curve for TPC determination

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).

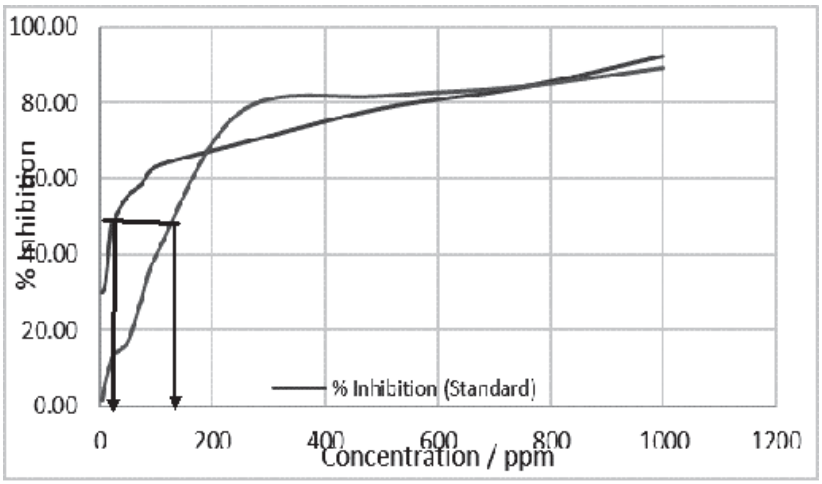

Figure 2. % Inhibition Vs concentration plot for leaves of *D. thwaites*

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

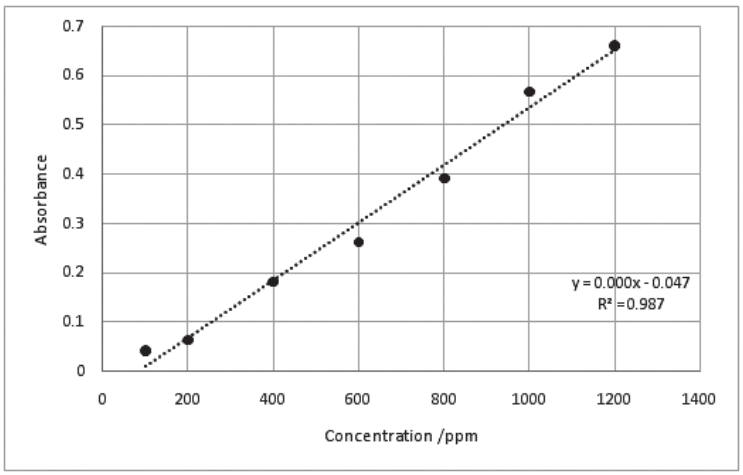

Figure 3. $FeSO_4.7H_2O$ standard curve for FRAP assay

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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