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ANALYSIS OF THE PHYTOCHEMICAL CONTENT AND DETERMINATION OF THE ANTIOXIDANT CAPACITY OF THE LEAVES, STEMS AND FLOWERS OF SESBANIA GRANDIFLORA (HUMMINGBIRD TREE) IN SRI LANKA USING DIFFERENT SOLVENTS FOR EXTRACTION

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ABSTRACT

Recently, plant-based medicines show growing demand as they are a source of natural antioxidants with great preference due to their minimized side effects. Sesbania grandiflora is rich in phytochemicals, and antioxidants, thus have been used in Ayurveda medicine. In the current study, the leaves, flowers, and stems of Sesbania grandiflora were manually extracted using 80% methanol, 80% ethanol, chloroform, and distilled water as solvents. Phytochemical screening tests were followed to confirm the occurrence of alkaloids, flavonoids, saponins, tannins, triterpenoids, cardiac glycosides, and carbohydrates. The antioxidant capacities were determined using the assays: 2.2' – Azino - bis (3 – ethylbenzothiazoline – 6 – sulfonic acid) (ABTS), 2,2 – Diphenyl – 1 – picrylhydrazyl (DPPH), Ferric-Reducing-Antioxidant-Power assay (FRAP), total antioxidant capacity assay (TAC), total flavonoid content (TFC), and total phenolic content (TPC). The spectrophotometric analysis was performed for determining the antioxidant capacity. All samples were triplicated. The leaves showed to contain a high concentration of the phytochemicals that were tested for, followed by moderate concentrations in flower and stem. The chloroform extracts of flower were shown to have more flavonoids (12.693 mgRE/g) while chloroform extracts of stems had more phenolic antioxidants (15.776 mgGAE/g). The results showed that chloroform is the most effective solvent to extract the non – polar phytochemicals in all three parts. The results of the DPPH, FRAP and ABTS assays demonstrated that methanol and ethanol extracts showed higher antioxidant activity. Furthermore, the methanol extracts of stem had stronger free radical scavenging activity in the DPPH (94%) assay, whereas ethanol stems had the most ABTS (81%) findings, and methanol flowers had the highest FRAP results (74 %). Further improvements to this research can aid in the development of new drugs that will work against many acute and chronic diseases.

Keywords: Sesbania grandiflora, Phytochemical content, Antioxidant activity

INTRODUCTION

Since ancient times, exploring nature particularly on plants has been taking place in search of new drugs (Kumar and Dhanyaraj, 2015). Medicinal plants play a major role in health care because they have been using to treat specific diseases or ailments all these years. Despite the recent advances in modern medicine, plant products still have an important contribution to healthcare (Gupta and Kishori, 2018). Increased demand for plant-based medicines leads to the use of a large number of medicinal plants with curative properties to treat a number of

diseases (Padmaja, Sravanthi and Hemalatha, 2011). Sesbania grandiflora is one of many herbal plants which have been studied for its immense contribution to curing diseases (Arun, 2011).

Figure 1: Leaves (A), stems (B), and flowers (C) of Sesbania grandiflora (Noviani et al., 2020)

Sesbania grandiflora (S. grandiflora) is a widely available herbal plant that belongs to the family Fabaceae and genus Sesbania (Arun et al., 2011). Which is a sparsely branching small tree that grows up to 15 m in height and 39 cm in diameter. This is also known as Kathurumurunga (Agathi) or Hummingbird tree (Okonogi et al., 2007). The native place of Sesbania grandiflora is tropical Asia and now it is widespread in Sri Lanka, Philippines, Indonesia, Malaysia, and India (Jiraungkoorskul and jiraungkoorskul, 2015). The herb contains unique medicinal properties as all parts are utilized as a medicine in Sri Lanka specifically leaves, flowers, and stems (Abubakar, 2015). Up to date reports stated that is S. grandiflora possesses antioxidant, antibacterial, antifungal, antiurolithiatic, anticonvulsant, antiarthritic, anti-inflammatory, anthelmintic, and anxiolytic activity (Mohiuddin, 2019; Ajayi et al., 2001; Arun et al., 2014)

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Table 1: Pharmacological activity and treated diseases by various parts of Sesbania grandiflora

Part of the plant	Formulation	Pharmacological activity
Leaves	Juices	Fever, leprosy, and worms (Neethu, 2016)
		Bronchitis, diarrhea, cough, and dysentery (Wagh, 2009)
	Tonic	Oral and throat infections (Bhoumik, 2016)
	Ethanolic extract	Antiulcer (Preclinical trials) (Bhoumik, 2016)
	Crushed leaves	Sprains and bruises (Kumar *et al*., 2010)
	Dried leaves (Powder)	Antitumor (Kasture, 2002)
	Aqueous extract	Anthelmintic (Kumar e*t al*., 2010)
	Ethanolic extract	Rheumatic swelling (Kasture, 2002)

Stems	Powder	Thrush and infantile disorders of the stomach (Anantaworasakul, 2017) Antibacterial activity (Eldeen, 2005)
Flowers	Powder	Diarrhea (Devasagayam *et al*, 2004)
	Ethanolic extract	Antidiabetic activity, Antiviral activity (Arthanari,2012)
	Methanolic extract	Anti-cancer (human colon cancer, Dalton lymphoma ascites) (Laladhas *et al*, 2010)

Previous studies have reported that Sesbania grandiflora is rich in various phytochemicals such as saponin, flavonoids, phenolics, alkaloids, tannins, carbohydrates, proteins, and glycosides which are pharmacologically active (Leitzmann, 2016; Arun et al., 2014). Secondary plant metabolites appear to play a critical role in human health and may be nutritionally significant, according to an increasing body of evidence, and screening of these phytochemicals can be considered as a valuable step in the detection of bioactive compounds and principles (Zhang et al., 2019). Phytochemicals are secondary metabolites derived from plants that have disease-preventive or defensive properties (Lillehoj et al., 2018). These are usually formed in very small amounts (Leitzmann, 2016). Their mode of action is either as antioxidants or as antimicrobials with the ability to kill bacteria (antibacterial activity). Alkaloids, carbohydrates, flavonoids, lipids, phenolic acids, terpenoids, and other nitrogen-containing metabolites are the most common phytochemicals identified (Akter et al., 2016). The predominant and most studied phytochemicals are polyphenols and carotenoids which play a major role in contributing to the antioxidant potential (Zhang et al., 2015; Liu, 2004). Since phytochemicals have fewer or no side effects, they are often used in the treatment of different diseases (Banu and Cathrine, 2015).

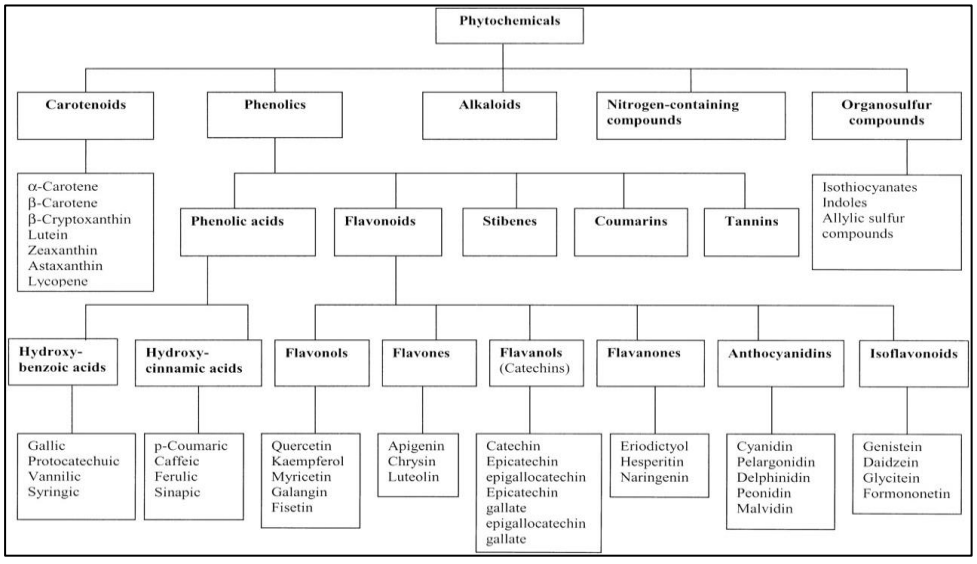


Figure 2. Classification of phytochemicals (Liu, 2004)

These phytochemicals may be either polar or non-polar. Figure 3 indicates that polar phytochemicals are soluble in polar solvents, whereas nonpolar phytochemicals are soluble to varying degrees in nonpolar solvents.

Phytochemicals	Ethanol extract	Chloroform extract
Sterols	(+)	(+++)
Triterpenes	(+++)	(-)
Flavonoids	(+++)	(++)
Alkaloids	(++)	(++)
Saponins	(+++)	(+)
Glycosides	(++)	(++)
Tannins	(+++)	(++)

(+) = traces, (++) = moderate, (+++) = abundant, (-) = absence of constituents

Figure 3. Phytochemical solubility in polar and non-polar solvents shows a vast degree (Dhayalan et al., 2018)

Past studies show that many phytochemicals act as antioxidants that contribute to neutralizing free radicals and removing their power to create damage (Zhang et al., 2015). Free radicals are chemical species that are associated with an unpaired electron that can exist independently (Valko et al., 2006). Highly active compounds can be formed when an interaction of oxygen occurs with certain molecules (Jacob, 2011). They are produced in the body as part of cellular respiration and have the ability to act as both oxidizing and reducing agents by donating and accepting electrons. (Lobo et al., 2010). Reactive oxygen species and reactive nitrogen species include several types of free radicals which are produced as products of the metabolization of molecular oxygen and nitrogen respectively (Laboni, 2016). Biochemical reactions result in the production of reactive oxygen species to maintain homeostasis and they act as signaling molecules in healthy tissues (Devasagayam et al., 2004). Under normal condition, there are various cellular functions which need free radical as essential such as transcription signal transduction and platelet adhesion (Buehler, 2012). However higher concentrations of the free radical are quite harmful to the body (Valko et al., 2006). Antioxidants are a group of molecules that could slow down or prevent the oxidation of other molecules to stop further production of free radicals (Jacob, 2011). Accumulation of reactive oxygen species or depletion of natural antioxidants leads to an imbalance between reactive oxygen species and antioxidants (Birben et al., 2006). Outweighing the production of antioxidants due to the accumulation of free radicals leads to a condition called oxidative stress (Valko et al., 2006).

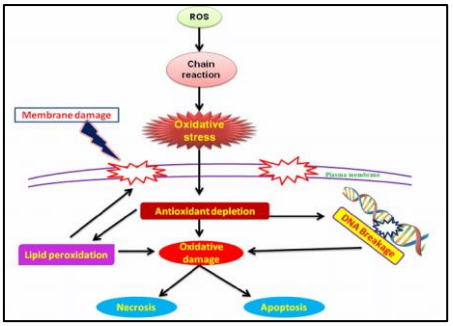


Figure 4: Schematic view of oxidative stress-induced cell tissue damage due to the depletion of the antioxidants (Finosh, 2013)

Oxidative stress will end up causing some acute or chronic diseases such as diabetes, hypertension, Parkinson's, and Alzheimer's (Liu et al., 2018). Furthermore, dietary supplements are used to consume synthetic antioxidants which help to improve oxidative stress (Stokes, 2020) while they are cheap and easy to process. It had been proven that synthetic antioxidants cause high cellular damage and toxicity compared to natural antioxidants (Valko et al., 2006). Natural antioxidants contained by many plants help to overcome all these devastating effects (Brewer, 2011). The extraction and characterization of several active antioxidants phytocompounds from green factories have resulted in the development of a number of drugs with high activity profiles (Akter et al., 2016). The first step is the extraction of desired active ingredients from the plant. According to the extraction principle, extraction methods include solvent extraction, distillation, pressing, and sublimation. Solvent extraction is the most widely used method. Any factor enhancing the diffusivity and solubility of phytochemicals in the solvent will facilitate the extraction (Zhang, Lin and Ye, 2018).

Table 2. Different conventional extraction (Solvent) methods along with the description on them (Swamy and Akhtar, 2019; Khan, Troquet and Vachelard, 2005).

Solvent extraction methods	Description
Maceration	Soaking the plant materials with a solvent in a sealed container and let for few days to obtain softer substances in a liquid medium
Percolation	A process of a liquid slowly passing through the filter to extract particular substances into the solvent
Soxhlet Extraction	Porous bag/thimble containing powdered sample is placed in a Soxhlet apparatus and solvents are vaporized onto the sample
Decoction	Hard plant materials are soaked in a specified volume of boiled water for a defined period
Infusion	Soaking plant materials in a specified volume of cold/boiled water for a shorter period

The percolation method takes very little time compared to the maceration technique while maceration does not require specific equipment and percolation requires specific equipment for extraction. Maceration, percolation, infusion, and decoction are known as the most common and easiest methods, and higher chances of presence of impurities and effect causes by choice of solvents are the disadvantages of these methods. Soxhlet method is the most efficient method which can produce accurate results but still, it is more time-consuming, and analysis of numerous samples is limited by the extraction step (Loganayaki, Suganya and Manian, 2012). For solvent extraction procedures several solvents are used to extract plant materials. The polarity of the solvent and its type affect the extract quality, quantity, extraction velocity, and antioxidant activity of phenolic compounds in plant material (Barchan et al., 2014; Zhang et al., 2019). During solvent extraction solubility of a solute is highly dependent on the polarity and dielectric constant of the solvent (Singh et al., 2014). A solvent with similar polarity to the solute will properly dissolve the solute/phytochemical (Polar phytochemicals dissolve in the polar solvent while non-polar constituents dissolve in non-polar solvents) (Altemimi et al., 2017). Methanolic and ethanolic extracts have been shown to produce higher yields of phenolic compounds in studies (Altemimi et al., 2017; Machmudah et al., 2017). Extracted phytochemicals can be used to analyze antioxidant capacity by using different assays.

Solvent	Solvent type	Dielectric constant
Water	Polar protic	80.1
Ethylene glycol	Polar protic	37.7
Glycerol	Polar protic	42.5
Ethanol	Polar protic	25
Methanol	Polar protic	33.6
Dimethylsulfoxide	Dipolar aprotic	48.9
Dimethylformamide	Dipolar aprotic	38.3
Acetone	Dipolar aprotic	20.7
Methylene chloride	Nonpolar	9.14
Benzene	Nonpolar	2.27
Toluene	Nonpolar	2.39
Hexane	Nonpolar	1.89

Figure 5. Different solvents and their respective dielectric constant values which decide polarity (Haidekker et al., 2005).

Antioxidant assays are important for determining the antioxidant capacities of medicinal plants in a high-throughput and cost-effective manner. Antioxidant capacities are currently calculated using a variety of assays, each with its own set of benefits and limitations (Bibi Sadeer et al., 2020). Antioxidant capacity is the measure of unknown antioxidant concentration by using spectrophotometry (Martono et al., 2019).

Table 3. Classification of antioxidant assays with few examples (Liang and Kitts, 2014)

Single-electron transfer measures (SET)/ Free radical scavenging assays	Antioxidant capacity assays
FRAP (Ferric ion reducing assay) DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) assay ABTS (2,2'-azino-bis 3-ethylbenzothiazoline-6-sulphonic acid) assay	TPC (Total phenolic content) TFC (Total flavonoid content) TAC (Total antioxidant capacity)

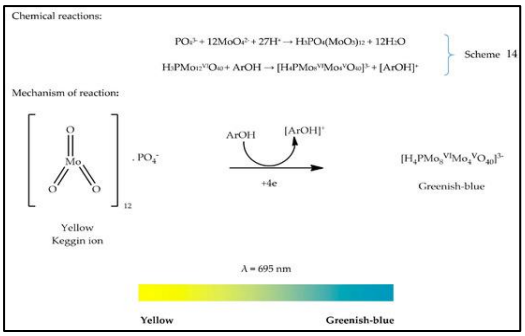


Figure 6. Principle of TPC assay (Ar- Aromatic rings) (Bibi Sadeer et al., 2020)

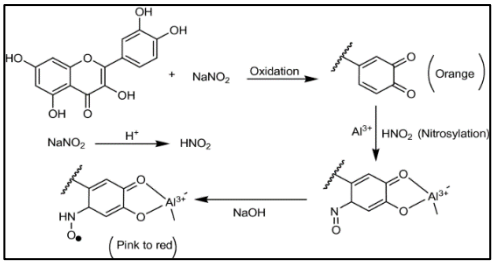


Figure 7. Principle of TFC assay (Mekkonan and Desta, 2021)

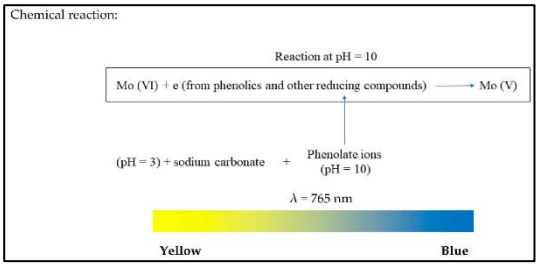


Figure 8. Principle of TAC assay (Bibi Sadeer et al., 2020)

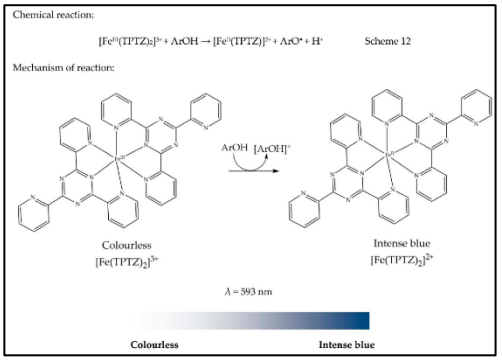


Figure 9. Principle of FRAP assay (Bibi Sadeer et al., 2020)

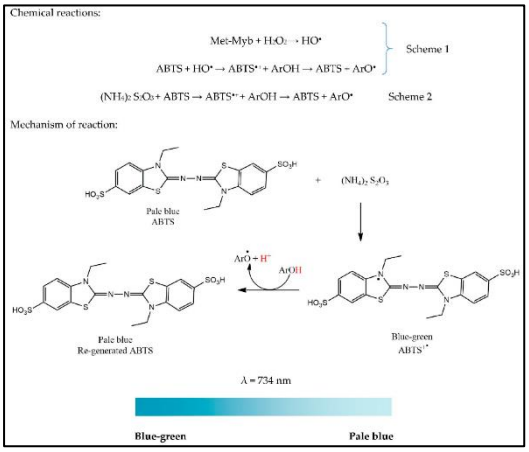


Figure 10. Principle of DPPH assay (HAT- hydrogen atom transfer) (Bibi Sadeer et al., 2020)

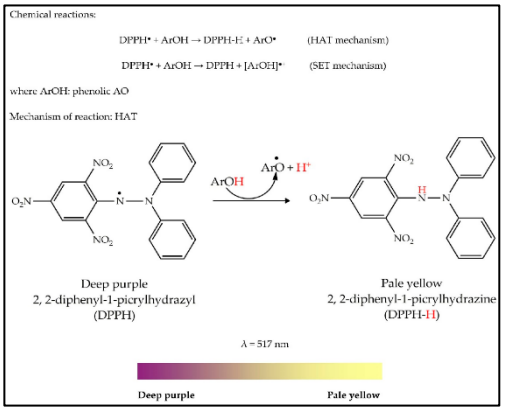


Figure 11. Principle of ABTS assay (Bibi Sadeer et al., 2020)

METHODOLOGY

Plant sample collection and preparation

Leaves, stems, and flowers used in the study were collected from open fields and households in Jaffna, Sri Lanka. Collected healthy plants were shade dried and powdered. Grounded samples were stored at 4ºC until the extraction process.

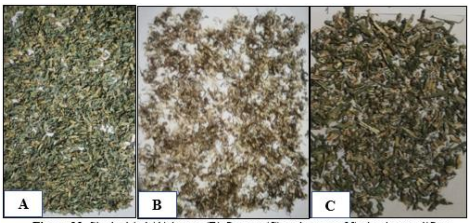


Figure 12. Shade dried (A) leaves (B) flowers (C) and stems of Sesbania grandiflora

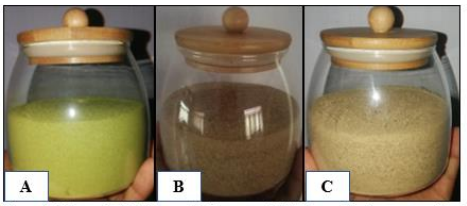


Figure 13. Grounded (A) leaves (B) flowers and (C) stems of Sesbania grandiflora

Sample extraction

Aliquots of 1.50g of each plant part were transferred into labeled 50ml falcon tubes. Then, 30.0 ml of each four solvents; 80% ethanol, 80% methanol distilled water (DW) and chloroform was transferred into their respective falcon tubes. Para filmed falcon tubes were kept on the roller mixer for 48 hours for maceration at 180 rpm. Later, the contents were filtered into new falcon tubes and Whatman No 1fillter paper to obtain the filtrate of solvent extracts (SE)/mother-solution of each sample (Neethu and Dhanyaraj, 2016).

Dilution of plant extracts filtrate

A dilution of 1:10 was done for each SE by adding 4.5 ml of respective solvent and 0.5 ml of plant extract. 80% methanol and 80% ethanol were prepared accordingly to the diluted samples.

Phytochemical screening tests

The phytochemical screening tests (PST) were carried out on diluted SEs to test for the presence of the phytochemicals; alkaloids (Padmalochana and Rajan, 2014) carbohydrates, flavonoids, Saponins (Arun et al., 2014), cardiac glycosides, tannins, and triterpenoids (Bahera, Karki and Shekar, 2012).

Table 8. Methodologies and expected results of PSTs

Phytochemical screening tests	Procedure
Alkaloids (Wagner's test)	Then into each test tube, 5.0 ml of SE (Already diluted as 1:10) and 1.0 ml of Wagner's reagent were added. Expected Result: Formation of the reddish-brown color precipitate
Flavonoids (Alkaline reagent test)	Into 5.0 ml of diluted solvent SE, few drops of 1.0 ml of 5% sodium hydroxide and few drops of diluted hydrochloric acid were added. Expected Result: Yellow color solution

Saponins (Froth test)	In this test, 5.0 ml of diluted SE was added into a test tube, and it was topped up with 5.0 ml of distilled water and test tubes were vigorously shaken. Expected Result: Presence of persistent foam
Tannins (Braymer's test)	Into 5.0 ml of diluted SE, 2.0 ml of 5% ferric chloride was added. Expected Result: Formation of the dark-blue color precipitate
Triterpenoids (Salkowski's test)	Into 5.0 ml of diluted SE, 1ml of concentrated sulfuric acid was added. Expected Result: Formation of the yellow color at the bottom
Cardiac glycosides (Keller Kiliani's test)	Into 5.0 ml of diluted SE, 1.0 ml of 5% glacial acetic acid and 1.0 ml of 1% of ferric chloride solution were added. After, 1.0 ml of concentrated sulfuric acid was added to the sides of the test tube using the graduated pipette. Expected Result: Formation of the brown ring at the interface
Carbohydrates (Molisch's test)	In to 5.0 ml of diluted SE, 1.0 ml of Molisch's reagent was added and mixed well. 1.0 ml of concentrated sulfuric acid was added along the wall. Expected results: red or violet ring formation

Dilution prior to the antioxidant assays

A dilution of 1:20 was done for each solvent extract.

Antioxidant Capacity assays

All the biochemical assays were carried out using spectrophotometry and the measurements were triplicated.

Total flavonoid content (TFC)

The methodology of TFC was adapted from Bao et al. (2005) and modified. Into 1.0 ml of diluted extracts, 300µl of 5% sodium nitrite was added. The solution was incubated for 5 minutes at room temperature (25ºC). After incubation, 300µl of 10% aluminum chloride was added and the solution was allowed for a further 5-minute incubation at room temperature. An aliquot of 4.0 ml of 4% sodium hydroxide and 400µl of DW was added further to top up the volume to 10.0 ml. Lastly, the tubes were kept for 15 minutes of incubation in a dark environment at room temperature. The absorbances of the samples were measured at 510 nm against the respective solvent blanks (80% Methanol, 80% Ethanol, Distilled water, and Chloroform). Then TFC was expressed in mg Rutin Equivalent (RE)/g of dried sample, using Rutin standard curve.

Total phenolic content (TPC)

The methodology of TPC was adapted from Matthaus, (2002) and modified. Into 1.2 ml of extract, 4.0 ml of diluted (1:10) Folin–Ciocalteu (FC) reagent was added, and which was followed by the addition of 4.8 ml of 7.5% sodium carbonate solution. Eventually, the tubes were incubated in kept in a dark environment for 30 minutes at room temperature. After incubation, the absorbances were measured at 740 nm against the respective solvents blank (80% Methanol, 80% Ethanol, Distilled water, and Chloroform). Then TPC was expressed in mg Gallic-Acid-Equivalents (GAE)/g of dried sample, using a gallic acid standard curve.

Total antioxidant capacity (TAC)

The methodology of TPC was adapted from Rajamanikandan et al. (2011) and modified as well. Into 1.5 ml of extract, 9.0 ml of TAC reagent was added and kept

in 95ºc water bath for 90 minutes incubation. After incubation, absorbances were measured at 695 nm as triplicates against the respective solvent blank. Then TPC was calculated in mg Ascorbic Acid Equivalents (AAE)/g of dried sample, using an ascorbic acid standard curve.

DPPH (2,2-diphenyl-1-picrylhydrazyl)-Radical-Scavenging

The methodology of the DPPH assay was adapted from Elansary et al. (2012) and was further modified. In a covered beaker, 36 mg of 0.1mM DPPH was dissolved with 150.0ml of absolute ethanol. Later, in the covered test tubes, 2.0 ml of solvent extract was mixed with 4.0 ml of DPPH reagent. Then the test tubes were incubated for 30 minutes in the dark. Sample and control (DPPH-reagent) absorbance were measured at 517 nm against methanol blank as triplicates. Then DPPH-Radical-Scavenging activity was expressed as percentage inhibition.

$$\%\text{Inhibition(Free radical scavenging activity)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

FRAP -Ferric-Reducing-Antioxidant-Power assay

The methodology of the FRAP assay was adapted from Adebiyi et al. (2017) and it was further modified. Aliquots of 5.0 ml of FRAP-reagent were preheated to 37ºc for 10 minutes and were added into 1.0 ml of diluted (20-fold) extract and incubated for 30 minutes at room temperature in a dark environment. Absorbance was measured at 595 nm against the control of ascorbic acid in FRAP reagent. Then FRAP was expressed as % inhibition.

$$\%\text{ Inhibition (Ferric reducing power)} = 1 - \left(\frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}}\right) \times 100$$

ABTS (2,2'-azino-bis)-Radical-Scavenging

The methodology of the ABTS assay was adapted from Dimitrova, Nedialkov and Kitanov, (2000) and it was further modified. An aliquot of 1.0 ml of plant extract was mixed with 1.0 ml of ABTS solution. The solution was kept under room temperature for 7 minutes as incubation. After incubation, the absorbance was measured at 734 nm against methanol blank as triplicates. Then ABTS-Radical-Scavenging activity was expressed as % inhibition.

$$\%\text{Inhibition(Free radical scavenging activity)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Statistical analysis

The data obtained for all of the assays was statistically analyzed using Microsoft-Excel software. To compare antioxidant capacity between Solvent extracts of plant parts, bar graphs were developed. These statistics revealed significant variations between the two sets of data. During the analysis mean-unknown-concentration, +/- standard deviation, standard error values were obtained.

RESULTS

Phytochemical screening test

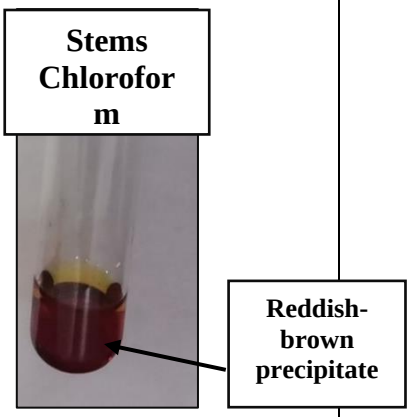
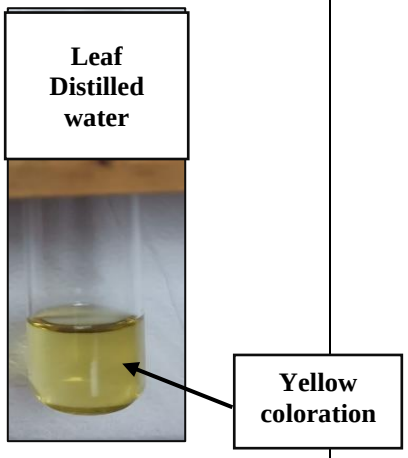
Table 9.0. Phytochemical screening test results of S. grandiflora extracts (Appendix-4.0.)

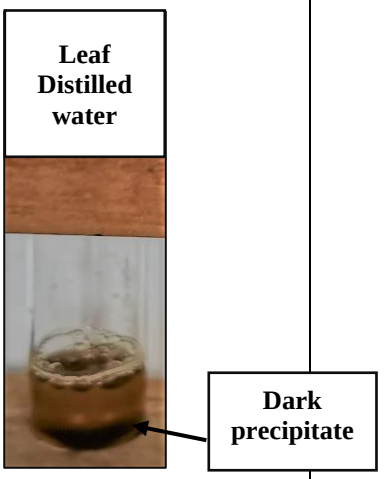
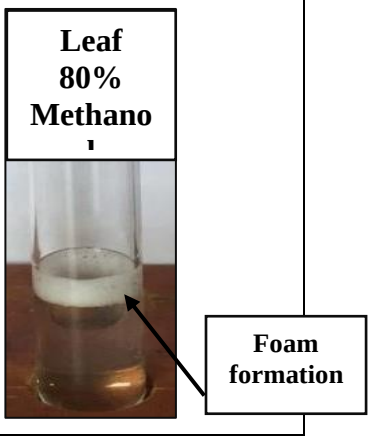
Phytochemical screening tests	80% Methanol			80%Ethanol			Distilled water			Chloroform		
	Leaf	Flower	Stem	Leaf	Flower	Stem	Leaf	Flower	Stem	Leaf	Flower	Stem
Alkaloids	√	√	√	×	×	×	√	×	×	√	√	√
Flavonoids	√	×	×	√	×	×	√	√	×	√	×	×
Tannins	√	×	×	√	×	×	√	×	×	√	×	√
Saponins	√	×	√	√	×	×	√	√	×	√	×	√
Triterpenoids	√	×	×	√	×	×	√	×	×	√	×	×
Cardiac glycosides	√	√	√	√	×	×	√	√	√	√	√	×
Carbohydrates	√	√	√	×	×	×	√	√	√	√	√	√

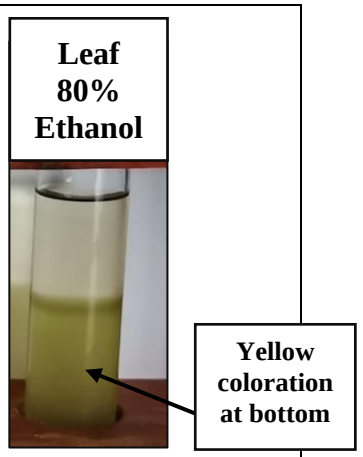
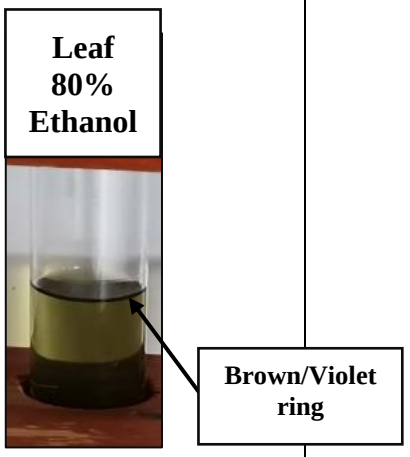
(√– Presence, × - Absence)

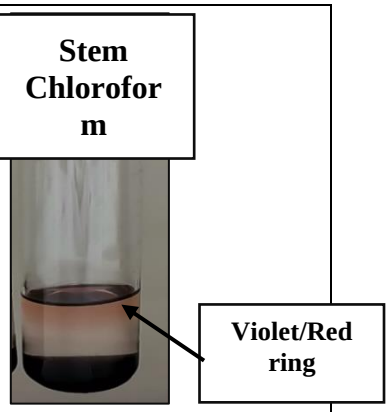
The highest occurrence of phytochemicals was observed in leaves with all the solvents used for the extraction while the lowest occurrence was observed in flower extracts. 80% ethanol extract of flower and stems resulted negative for all the phytochemical screening tests while methanol, ethanol, and chloroform extracts of leaves sample confirmed the presence of all the tested phytochemicals. Stems extracts of all four solvents resulted positive for a moderate amount of PSTs.

Table 10.0. Reference of positive results along with the tests

Tests	Results
Alkaloids	
Flavonoids	

Tannins	
Saponins	

Triterpenoids	
Cardiac glycosides	

Carbohydrates	

Antioxidant assay results

Total Flavonoid Content (TFC)

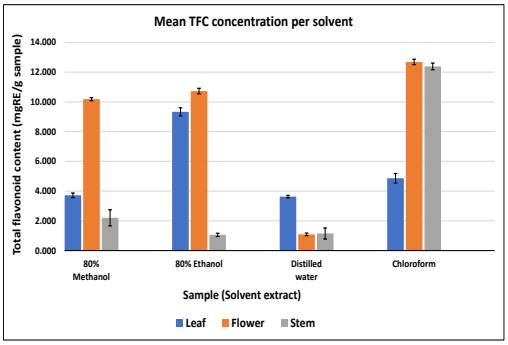


Figure 14.0. Bar chart with error bars for mean TFC concentration of 80% ethanol, 80% methanol, distilled water, and chloroform of leaf, flower & stem solvent extracts.

Chloroform flower extracts resulted in the highest TFCs while the lowest value was observed in DW flower extracts. Flower extracts of methanol, ethanol, and chloroform resulted in the highest TFCs when compare with the leaves and stem extracts of these respective solvents.

Total Antioxidant Content (TAC)

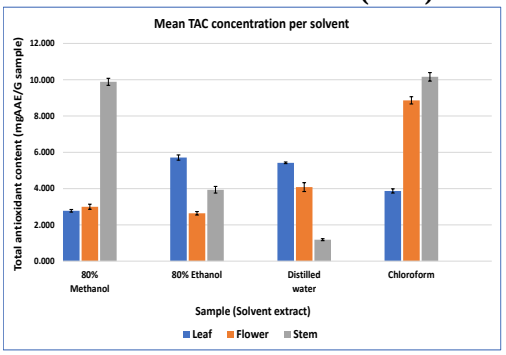


Figure 15.0. Bar chart with error bars for mean TAC concentration of 80% ethanol, 80% methanol, distilled water and chloroform of leaf, flower & stem solvent extracts.

Chloroform stem extracts resulted in the highest TACs while the lowest value was observed in DW stems extracts. Methanol and chloroform stem extract, ethanol, and DW leaves extracts were exhibited the highest TACs when compare with the rest of the sample extracts of the respective solvents

Total Phenolic Content (TPC)

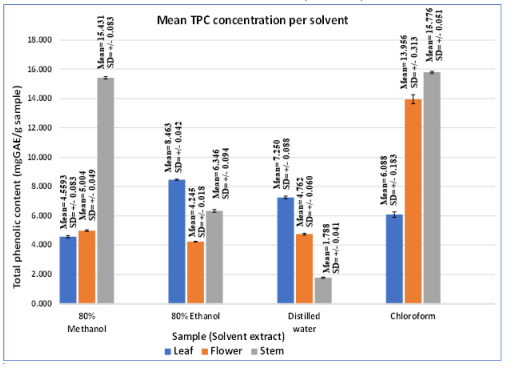


Figure 16.0. Bar chart with error bars for mean TPC concentration of 80% ethanol, 80% methanol, distilled water, and chloroform of leaf, flower & stem solvent extracts.

The highest TPC was observed in chloroform stem extracts while the lowest was observed in distilled water stems extracts. Methanol and chloroform stem extract, ethanol and DW leaves extracts were exhibited the highest TPCs when compare with the rest of the sample extracts of the respective solvents.

DPPH assay

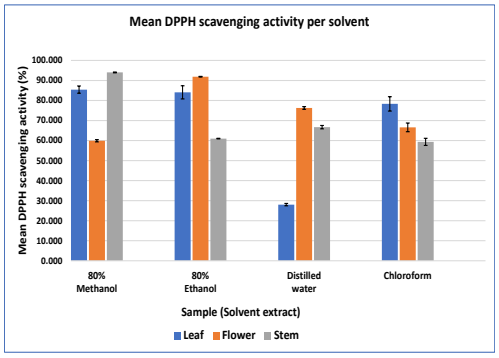


Figure 17.0. Bar chart with error bars for mean DPPH % scavenging activity of 80% ethanol, 80% methanol, distilled water, and chloroform of leaf, flower & stem solvent extract

The highest DPPH % scavenging activity was obtained in 80 % methanolic extracts of stems (94.032%) while distilled water leaves extracts resulted in the lowest DPPH % scavenging activity (65.111%). Flower extracts of 80 % ethanol and DW

resulted in the highest DPPH % scavenging activity when compared with leaves and stem extracts of the respective solvents. The highest DPPH % scavenging activity amongst all three extracts of methanol was observed in stem extract while leaves extracts of chloroform resulted in the highest amongst all three sample extracts of chloroform.

ABTS assay

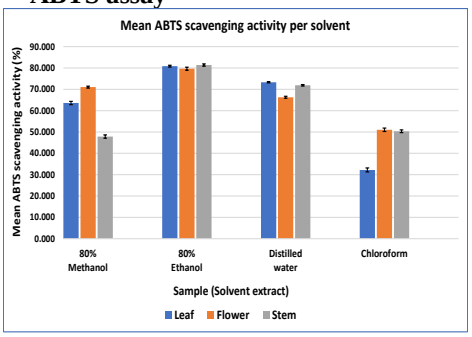


Figure 18.0. Bar chart with error bars for mean ABTS % scavenging activity of 80% ethanol, 80% methanol, distilled water, and chloroform of leaf, flower & stem solvent extracts.

The highest ABTS % scavenging activity was obtained in 80 % ethanolic extracts of stems (81.387%) while chloroform leaves extracts resulted in the lowest ABTS % scavenging activity (32.194%). Flower extracts of 80 % methanol and chloroform resulted in the highest ABTS % scavenging activity when compare with leaves and stems extracts of the respective solvents.

FRAP assay

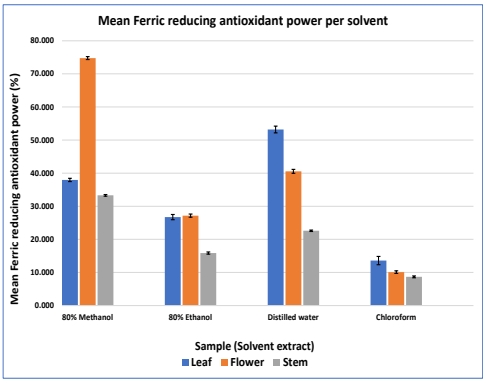


Figure 19.0. Bar chart with error bars for mean FRAP % scavenging activity of 80% ethanol, 80% methanol, distilled water, and chloroform of leaf, flower & stem solvent extracts.

Methanol extracts of flowers derived the highest (74.770%) FRAP % scavenging activity and chloroform extracts of stems resulted the lowest (8.683%) FRAP % scavenging activity. Leaves extracts of chloroform and DW showed high FRAP % scavenging activity. Flowers extracts of methanol and ethanol showed high FRAP % scavenging activity.

DISCUSSION

Sri Lanka has a long tradition and history of using indigenous medicine. Plant products have been used in Sri Lanka for centuries to treat a variety of diseases (Okonogi et al., 2007). However, the growth of Western medicine took away the scope and attention of indigenous medicine but recently, the increased drug abuse and antibiotic resistance leading to the regeneration of natural medicine using herbs in Sri Lanka. Therefore, it is considered as important to identify and quantify the beneficial properties of the medicinal plants used in Sri Lanka. In Sri Lanka, S. grandiflora (Kathurumurunga) has been used as a medicinal plant for centuries (Gunathilake and Ranaweera, 2016). However, there is not much published research available on antioxidant properties and phytochemical analysis of different parts of the Sesbania grandiflora plant in Sri Lanka. Gunathilake and Ranaweera (2016) analyzed the antioxidative properties of 34 different green leafy vegetables with the inclusion of Sesbania grandiflora and their study mainly analyzed the antioxidant properties of leaves of 34 different herbs and there was no comparison between different parts or usage of different solvent extraction in the research.

This study was done to evaluate the most effective solvent to extract the phytochemicals in different parts of Sesbania grandiflora and to determine the relationship between antioxidant properties and free radical scavenging activity. Further, an abundance of phytochemicals in different parts of Sesbania grandiflora can also be analyzed through the comparison between antioxidant assay results of respective plant parts. Moreover, obtained results allow us to understand the essential properties of this herbal plant. The leaves, flowers and stems were chosen to carry out the study as leaves and flowers possess significant antioxidant activity while there is limited evidence for the activity of the stems (Bahera, Karki and Shekar, 2012). Because of the wide range of bioactive compounds found in plant materials and their varying solubility properties in various solvents, the best solvent for extraction is determined by the specific plant materials. As a result, selecting an appropriate extraction solvent for specific plant materials is typically difficult (Truong et al., 2019). Methanol has been found to be more effective at extracting lower molecular weight polyphenols, while chloroform is better at extracting higher molecular weight flavanols. (Do et al., 2014). 80% methanol, 80% ethanol, distilled water, and chloroform were chosen as solvents during this study in order to do a comparison study on the effectiveness since all four solvents have different polarity and dielectric constants which contribute to extraction yield.

The abundance of phytochemicals can be affected significantly by drying the sample (Nurafifah et al., 2018). The samples in this study were shade-dried, while other drying methods, such as freeze-drying, have been shown to be successful in extracting phytochemicals, indicating a significant impact (Bhatt and Negi, 2012). The manual extraction method was used in this study instead of automated extraction methods such as centrifugation. As a result, the extracted sample was not 100 % pure. Traditional techniques such as centrifugation and the Soxhlet apparatus can be used to achieve a

more purified sample. Since the Soxhlet apparatus is restricted for lipid extraction and centrifugation is time-consuming, alternative methods such as pressurized – liquid extraction and microwave-assisted extraction (MAE), which are more advantageous than the traditional process, can be used (Azmir et al., 2013). It was also found that MAE aids in the extraction of phytochemicals, resulting in increased antioxidant activity. This has also been shown to reduce extraction time and solvent consumption. (Li et al., 2012).

According to the obtained results of PSTs, which are shown in Table 9.0, it can be concluded as follows. Methanolic, distilled water, and chloroform extracts of leaves resulted positive for all seven phytochemical screenings (Alkaloids, Flavonoids, tannins, saponins, cardiac glycosides, triterpenoids, and carbohydrates) and which confirms their presence in leaves of S. grandiflora. Alkaloids and carbohydrates resulted negative for ethanolic extract while the presence of the rest of the six phytochemicals was confirmed. On close analysis and appraisal, we see those leaves of S. grandiflora contain all the tested phytochemicals. This is supported by the studies done by Arun et al. (2014); Reji and Alphonse, (2013); Kumar et al. (2010). The PSTs of methanolic and chloroform extracts of flowers confirm the presence of alkaloids, cardiac glycosides, and carbohydrates while distilled water extracts resulted positive for saponins, flavonoids, cardiac glycosides, and carbohydrates. None of the PST resulted positive for ethanolic extracts of flowers. This confirms that the flower of Sesbania grandiflora contains alkaloids, flavonoids, saponins, cardiac glycosides, and carbohydrates while they do not consist of triterpenoids, and tannins. This is supported by the research done by Bahera, Karki and Shekar, (2012); Abubakar et al. (2015).

Ethanolic extracts of stems resulted in all negative for PST. Cardiac glycosides and carbohydrates are present in distilled water extracts. Only saponins, tannins, alkaloids, and carbohydrates extracted by chloroform and methanol. As a brief, S. grandiflora stem only contains few tested phytochemicals, saponins, tannins, alkaloids, cardiac glycosides, and carbohydrates. This is also supported by Divya et al, (2018). The different extracts of the leaves and flowers have clearly indicated that all the major phytochemicals (Alkaloids, Flavonoids, saponins, cardiac glycosides, and carbohydrates) are present in the extracts and so these plant leaves and flowers can be used perfectly as a major part for extraction of bioactive compounds. Phytochemical contents of Sesbania grandiflora stems seem relatively less than leaves and flowers. Since methanol extracted the majority of the phytochemicals, it can be considered as the most efficient solvent, indicating that methanol is used as a solvent in Ayurveda centers for extracting bioactive compounds (Arun et al., 2014). DW and chloroform resulted in a moderate amount of effectiveness. 80% ethanol can be considered as the least effective solvent for this particular plant phytochemical extraction since it resulted in very low effectiveness. Flavonoids and phenolics are two important plant compounds that are known for their antioxidant properties (Senguttuvan, Paulsamy and Karthika, 2014). Phenolics are a broad group of phenol-derived phytochemicals with a variety of properties, including the ability to prevent ulcer formation and angiogenesis (John et al., 2014). Flavonoids are a subcategory of the phenolic compound that has a lot of medicinal properties (John et al., 2014; Saxena et al., 2013). Hence, it would be beneficial to quantify the amount of phytochemicals present in order to

understand the antioxidant activity produced (Pontis, 2014).

Antioxidant capacity assays, which measure the unknown concentration by absorbance of sample color changes, were used to confirm the most effective solvent for extracting phytochemicals by drawing quantitative results. In TFC-assay, the formation of colored complexes was assessed by the metal-ion-chelation-reaction of Al3+ on flavonoids (Martono et al., 2019). According to figure 14.0, results of TFC assays prove that chloroform is the most effective solvent to extract the highest concentration of flavonoids while ethanol is the least effective solvent, and this is supported by the PST results because chloroform extracts of leaves show the occurrence of flavonoids in PSTs. Flower extracts of all four solvents showed Similar results were obtained by Kumar and Dhanyaraj, (2015). It can also be predicted that most of the flavonoids are non-polar in nature in S. grandiflora samples and this is caused for the higher TFC with chloroform extracts. Since the error bars of 80% methanol and DW leaves extract overlap, it indicates no significant difference between the TFC concentrations in ethanol and DW leaves extracts, but error bars of leaves extract of 80% ethanol and chloroform do not overlap which indicates the presence of a significant difference between the TFC concentrations of those two. Collectively these results indicate that both 80% ethanol and DW are equivalently effective in the extraction of flavonoids from leaves. Flower extracts of all four solvents showed a significant difference between the TFC concentrations. No significant difference between the TFC concentrations in 80% ethanol and DW stems extracts observed while stems extract of 80% methanol and chloroform showed a significant difference between the TFC concentrations. Collectively these results indicate 80% methanol and DW are equivalently effective in the extraction of flavonoids from stems.

The TAC assay works on the basis that any phenolic compounds present in the sample will react with the FC reagent and produce a blue solution and this helps to measure the amount of phenol (Pontis et al., 2014; Everette et al., 2010). According to figure 15.0, results prove that chloroform and 80 % methanol are the most effective solvents to extract the highest concentration of antioxidants. This can be further supported with PST results and all the phytochemicals that can be considered as antioxidants (Alkaloids, flavonoids, triterpenoids, tannins, and saponins) showed positive results in chloroform extracts. In addition, it can be assumed that all three samples of this plant contain nonpolar antioxidants abundantly and which resulted in higher antioxidant capacity with chloroform extracts. Error bars of all four solvent extracts of leaves, flowers and stems do not show overlap and this indicates a significant difference between TAC concentrations of all the respective extracts of different solvents. The reduction of transition-metal-ion/molybdenum center; from Phosphate Mo (VI) to phosphate Mo (V) by electron-donating Phenols to form blue-colored complexes was assessed during the TPC assay (Gupta, 2015; Phatak and Hendre, 2014). According to figure 16.0, results prove that chloroform and 80 % methanol are the most effective solvents to extract the highest concentration of antioxidants. Error bars of all four solvent extracts of leaves, flowers, and stems do not show overlap and this indicates a significant difference between TPCs of all the respective extracts of different solvents.

DPPH is a purple free radical which gets reduces in the presence of phytochemicals/antioxidants of plant extracts and gives colorless DPPH.H (Gupta, 2015; Senguttuvan, Paulsamy and Karthika, 2014). DPPH assay results in figure 17.0 show the lowest amount of

antioxidants remain in 80%-methanol stem extracts after most of its antioxidants were used up to scavenge the DPPH. Since 80% methanol extracts resulted highest DPPH % scavenging activity, it can be considered as most effective solvent and similar results were obtained by Siddhuraju et al., (2014). External studies mention, the greater the % scavenging activity by DPPH, the lower remaining concentration of plant extracts and this supports the current research statistics (Nambirajan et al., 2018). Error bars of 80 % methanol and 80 % ethanol leaves extracts show overlap, and this indicates there is no significant difference on % scavenging activity of those two solvents, and which proves methanol and ethanol are equivalently effective when it comes extraction of antioxidants from the leaves sample. Further 80 % ethanol and chloroform extracts of stems also have overlap and this can be concluded as both solvents are equivalently effective when it comes extraction from the stem samples. The rest of the solvent's extracts show no overlap, and this indicates a significant difference between solvent extracts.

When compared to DPPH, the ABTS assay is normally effective at detecting antioxidant activity because it has faster reaction kinetics and a higher sensitivity to antioxidants (Lee et al., 2015). Other benefits of the ABTS assay include lower cost, time, and sample length (Moniruzzaman et al., 2011). The decolorization of the sample in the ABTS assay was due to the stabilization of ABTS monocation/free-radical in plant extracts by hydrogen-donating antioxidants (Kumaran and Karunakaran, 2007). ABTS assay results in figure 18.0 show that 80%-ethanol is the most effective solvent which could extract enough antioxidants to scavenge ABTS-free-radical. These results are supported by Siddhuraju et al., (2014). Error bars of all four solvent extracts of leaves, flowers, and stems do not show overlap and this indicates a significant difference between ABTS % scavenging activity of all the respective extracts of different solvents. The FRAP assay works on the principle that antioxidants form complexes with metal ions, as in the case of phosphomolybdenum (Vijayalakshmi and Ruckmani, 2016). FRAP assay results in figure 20.0 show that 80% methanol is the most effective solvent which could extract enough antioxidants to scavenge the free radical. These results are supported by Siddhuraju et al., (2014). Error bars of all four solvent extracts of leaves, flowers, and stems do not show overlap and this indicates a significant difference between ABTS % scavenging activity of all the respective extracts of different solvents.

CONCLUSION

Based on the results of the current study it can be concluded that Sesbania grandiflora leaves contain all the tested phytochemicals and methanol is the most effective solvent to extract all the tested phytochemicals. Antioxidant assay results clearly demonstrated that chloroform is the best solvent to extract a higher concentration of antioxidants from all three parts of the plant and this proves that non-polar antioxidants (non-polar flavonoids and phenolics compounds) are abundant in all three samples (leaves, flowers, and stems) of S. grandiflora. Results demonstrated that flowers contain more flavonoids (TFC) while stems of the plant contain a high level of phenols (TPC) than the other tested parts. Collectively it is proved that stems of the plant have the higher antioxidant capacity (TAC). Free radical scavenging activity assay (DPPH, FRAP and ABTS) results demonstrated that methanol and ethanol are the best solvents to extract antioxidants which shows higher free radical scavenging activity. In addition, leaves samples resulted in higher free radical scavenging activity as well. Many more experiments

can be conducted on this plant where the plant can be grown at different conditions and checked assayed for the phytochemicals. As future works, a comparison study can be done between different maturation stages of S. grandiflora leaves, flowers, and stems since the levels of phytochemicals increase with the maturation stage of plant parts. In addition, seeds can be compared with other samples. Different species of plant parts and different varieties of S. grandiflora flowers (pink, red and white) also can be compared in future studies using High Performance Liquid Chromatography/Mass Spectrometry (HPLC/MS) and Nuclear Magnetic Resonance Spectroscopy (NMR) are recommended to be examine. Other modifications like boiling the samples at various temperatures before extraction could be a possible evaluation. Tests can be developed to detect antibacterial properties of S. grandiflora leaves and bark and statistical analysis can be performed to find the correlation between the antioxidant capacity and the antibacterial properties which can be aided in future drug discovery and pharmaceutical applications of the industry

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