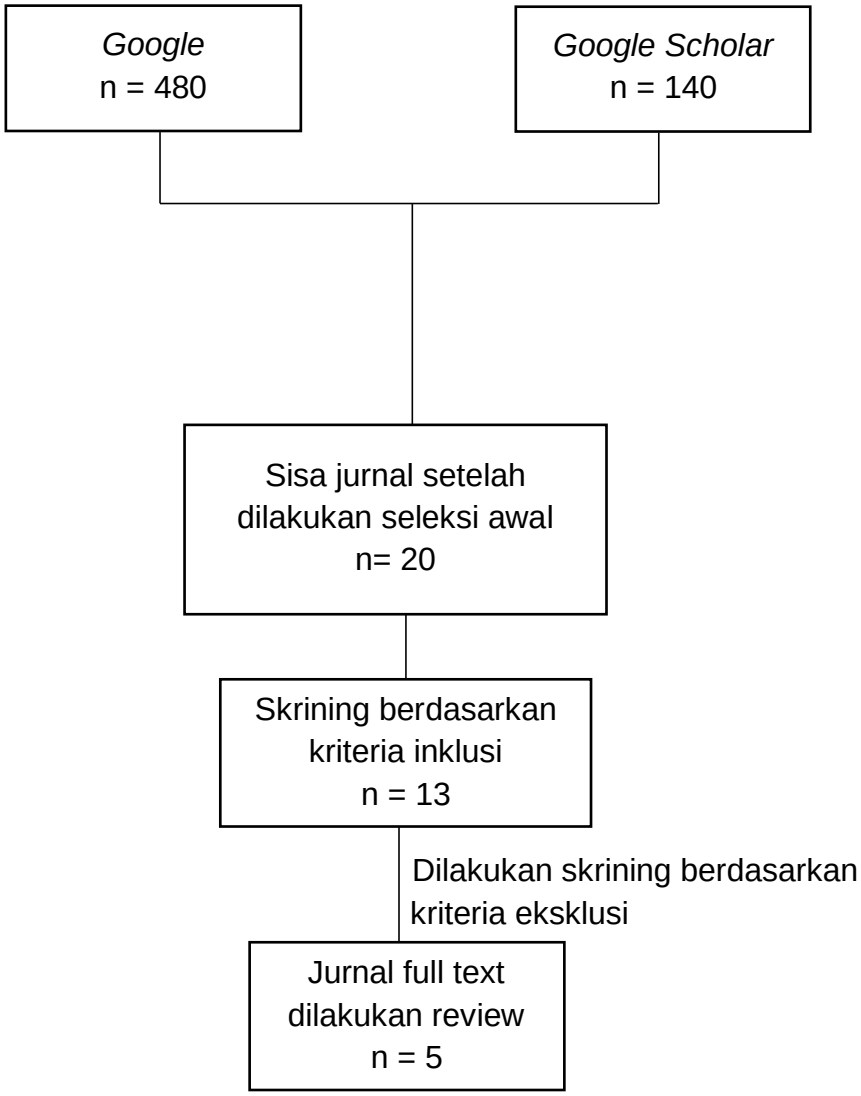
LAMPIRAN

Lampiran 1. Proses Pemilihan Literatur



Lampiran 2. Gambar Daun Turi *(Sesbania grandiflora L)*

Gambar 1. Tanaman Turi (*Sesbania grandiflora* L.)

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Response Surface Optimization of Extraction of Polyphenols and Carotenoids from *Sesbania grandiflora* Leaves with Ethanol-water System

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Authors' contributions

This work was carried out in collaboration between all authors. Authors KDPPG, KKDSR and HPVR designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors ODANP and HPSJ managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Response surface methodology was employed to optimize the extraction parameters for the extraction of total phenolics and carotenoids from leaves of *Sesbania grandiflora* with ethanol-water based system.
Method: The effects of solvent concentration (30-100%), extraction temperature (30-60°C) and

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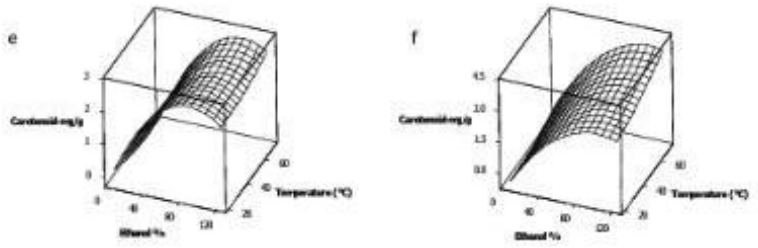

Fig. 2. Pair wise response surface plots of the carotenoids (mg/g DW) extraction from *Sesbania grandiflora* leaves as a function of ethanol %, extraction temperature and time: ethanol % was kept constant at 30% (a) and 100% (b); temperature of extraction was kept constant at 30°C (c) and 60°C (d); the time of extraction was kept constant at 30 min (e) and 90 min (f)

Table 5. Predicted values and experimental values of total phenolics and carotenoids at the optimum extraction conditions for *Sesbania grandiflora*

Optimum extraction conditions		Predicted values (mg/g)		Experimental values (mg/g)	
Phenolics	**Carotenoids**	**Phenolics**	**Carotenoids**	**Phenolics**	**Carotenoids**
ETOH:46.6%	ETOH:100%	7.74	4.32	8.09±1.35	5.12±0.93
Temp:70.2°C	Temp:70.2°C				
Time:110.5 min	Time:110.5 min				

3.4 Optimization of Phenolics and Carotenoids and Verification of the Model

Multiple graphical and numerical optimizations were run for determining the optimum levels of studied extraction conditions with desirable levels of phenolics and carotenoids contents. Optimum ethanol concentration, extraction temperature, extraction time were developed for the two responses and they were 46.6%, 70.2°C and 110.5 min for phenolics and 100%, 70.2°C and 110.5 min for carotenoids, respectively (above Table 5). For these optimum extraction conditions, the corresponding predicted response values for phenolics and carotenoids were 7.74 mg GAE/g DW and 4.32 mg/g DW, respectively. An experiment was run in accordance with the recommended optimum conditions for two responses, phenolics and carotenoids. More interestingly, in this study, the values obtained experimentally for both response variables are near to the predicted values, indicating a satisfactory model. The experimental values for total phenolics were 8.09 ± 1.35 mg GAE g extract and 5.12 ± 0.93 mg/g DW carotenoids and no significant difference ($p > 0.05$) was found between the experimental and predicted values of the extractable phenolics and carotenoids from leaves of *S. grandiflora* extract. Therefore, the data confirm the validity of the optimized model.

4. CONCLUSIONS

RSM was successfully implemented to optimize the total phenolics and carotenoid extraction from leaves of *S. grandiflora*. Overall, phenolic extraction prefers low ethanol concentration and the effect of temperature and extraction time was insignificant ($p>0.05$) for total phenolic extraction. Higher carotenoid recovery was observed at higher ethanol concentrations and lower extraction temperatures. Optimum ethanol concentration, extraction temperature and time were 46.6%, 70.20°C, 110.45 min for phenolics and 100%, 70.2°C and 110.5 min for carotenoids respectively. Results revealed that there were no significant differences between the predicted values for studied responses and experimental values obtained with optimum extraction conditions.

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Antioxidant Capacity and Total Phenolic Content of Aqueous Acetone and Ethanol Extract of Edible Parts of *Moringa oleifera* and *Sesbania grandiflora*

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***Abstract*—**Aqueous ethanol and aqueous acetone extracts of *Moringa oleifera* (outer pericarp of immature fruit and flower) and *Sesbania grandiflora* white variety (flower and leaf) were examined for radical scavenging capacities and antioxidant activities. Ethanol extract of *S. grandiflora* (flower and leaf) and acetone extract of *M. oleifera* (outer pericarp of immature fruit and flower) contained relatively higher levels of total dietary phenolics than the other extracts. The antioxidant potential of the extracts were assessed by employing different *in vitro* assays such as reducing power assay, DPPH˙, $ABTS^{\cdot+}$ and ˙OH radical scavenging capacities, antihemolytic assay by hydrogen peroxide induced method and metal chelating ability. Though all the extracts exhibited dose dependent reducing power activity, acetone extract of all the samples were found to have more hydrogen donating ability in DPPH˙ (2.3% - 65.03%) and hydroxyl radical scavenging systems (21.6% - 77.4%) than the ethanol extracts. The potential of multiple antioxidant activity was evident as it possessed antihemolytic activity (41.2 % to 68.0 %) and metal ion chelating potency (45.16 - 104.26 mg EDTA/g sample). The result indicate that acetone extract of *M. oleifera* (OPIF and flower) and *S. grandiflora* (flower and leaf) endowed with polyphenols, could be utilized as natural antioxidants/nutraceuticals.

***Keywords*—**Antioxidant activity, *Moringa oleifera*, Polyphenolics, *Sesbania grandiflora*, Underutilized vegetables.

I. Introduction

FREE radicals are naturally produced in the body through normal metabolism of biomolecules like carbohydrates, amino acids and fats. Over production of free radicals cause oxidation of these biomolecules which can lead to a variety of diseases such as cancer, cardiovascular diseases, cataracts, diabetes, and inflammatory diseases [1] along with induces deterioration of food, resulting in rancidity, changes in color, and declines in nutritional quality, flavor, texture and safety. Antioxidants are chemical compounds that can bind to free oxygen radicals and preventing these radicals from damaging healthy cells and it can be added to food products especially lipid containing food to increase the shelf life of foods [2]. Commonly used synthetic antioxidants like butylated hydroxyanisole (BHA) and butylated hydroxytolune (BHT) are suspected to cause some safety concerns because their consumption may cause cancer and liver damage. Therefore, the search for alternative sources of natural antioxidant is becoming increasingly important. The various antioxidant content such as phenolic compounds, nitrogen compounds, vitamins, and some other endogenous metabolites occur naturally in edible plants including fruits, vegetables, legumes, spices, and medicinal herbs. The presence of wide variety of biologically active, non-nutritive compounds like phytochemicals in the fruit and vegetables which impart health benefits beyond basic nutrition and it also prevents the some chronic diseases including coronary heart diseases, certain cancers and diabetes [3]. Leafy vegetables are considered to be a valuable part of the diet owing to their nutritive values which plays an important role in the human diet. Green leafy vegetables, some of which, underexploited, are proven significant sources of essential nutrients including vitamins, carotenoids, flavonoids, phenolics etc. [4]. Leafy vegetables are rich sources of minerals along with, antioxidants and pigments. The regular consumption of such leafy vegetables significantly plays an important role in maintaining a balanced diet and helps to avert the chronic effects of malnutrition.

Sesbania grandiflora belonging to Leguminosae family is comprised of about 60 species which are distributed throughout tropical and subtropical regions. *S. grandiflora* is a loosely branching tree up to 15 m tall, leaves and flowers are used as food, traditional medicine [5] and also used for soil enrichment and as animal forage. All parts of this plant such as bark, leaves, flowers, fruit and seeds contains plenty of sterols, saponins and tannins which are responsible for the treatment of multifactorial diseases [6]. The flowers are pink, red or white in color and are consumed in India as vegetables. Consumption of those flower vegetables can cure illness and disease such as diarrhea, stomach ache and nausea because of having the activity against pathogenic bacteria [7]. Active ingredients like leucocyanidine and cyanidine are found in seeds, oleanolic acid and its methyl esters and kaemferol-3-rutinoside found in flowers and tannins and gums are present in bark [8], and leaves contains rich sources of amino acids, minerals and vitamins like vitamin A, C, and B complex [9]. The leaves are used as aperients, diuretic and tonic and applied to bruises. Barks are astringent preferentially used in the treatment of small pox and other eruptive fevers. All parts of this plant are used as important nutritive source in Southeast Asian countries. In Ayurvedic system the fruit of this plant is prescribed for anemia, bronchitis, fever, pain, thirst, ozoena and Quartan fever [10]. *S. grandiflora* exerts its protective action against leprosy, gout, rheumatism, cancer, liver disorders [11], hypolipidemic [9], ulcer [12], urolithiatic [13], and hepatoprotection [14].

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extracts of *M. oleifera* and *S. grandiflora* was estimated by Yamaguchi et al. [45] and the results are present in Table II. All the extracts demonstrated the ability to chelate irons and 70% acetone extract of *M. oleifera* (OPIF) (104.26 mg/g), flower (97.38 mg/g) and *S. grandiflora* (flower) (57.45 mg/g), leaf (48.84 mg/g) were found to be higher than chelating activity of ethanolic extract of respective samples (51.55 mg/g, 56.90 mg/g, 49.19 mg/g, 45.16 mg/g). The present report showed higher chelating ability than the leaf, stem and flower extract of *Myrtus communis* var. *italica* L. [61]. From the result it was evident that both extract of *M. oleifera* and *S. grandiflora* able to play a protective role against oxidative damage by metal catalyze Fenton-type reaction. The metal chelating properties of the additives may be attributed to their endogenous chelating agents, mainly phenolics. Chelating agents may serve as secondary antioxidants because they reduce the redox potential thereby stabilizing the oxidized form of the metal ions. Our results suggest that the ferrous ion chelating effects of these fractions would be highly beneficial to protect against oxidative damage.

G. Hydroxyl Radical Scavenging Activity

Hydroxyl radical is considered to be a highly potent oxidant formed in biological systems and has been concerned as a highly damaging species in free radical pathology, capable of damaging almost every molecule found in living cells. This radical has the capacity to conjugate with nucleotides in DNA, cause strand breakage, and lead to carcinogenesis, mutagenesis and cytotoxicity [62]. Thus, removing hydroxyl radical is very important for the protection of living systems. The hydroxyl radical scavenging potential of an extract is directly related to its antioxidant activity. The hydroxyl radical scavenging activity of the two different solvent extract of the *M. oleifera* (OPIF and flower) and *S. grandiflora* (flower and leaf) is shown in Fig 5. The range of hydroxyl radical scavenging activity was 21.6-82.4% and the order of activity was CAT> MFA> MPA> SLWA> SFWA> MPE> MFE> SFWE> SLWE. At 250 µg, acetone extract of *M. oleifera* (OPIF and flower) and *S. grandiflora* (flower and leaf) exhibited higher radical scavenging activity than the methanolic extracts. Similarly, a highly potential hydroxyl radical scavenging was observed for leaf extract of *M. oleifera* [63], *S. grandiflora* [64] and polysaccharide from flower of *Camellia sinensis* [65]. The ability of the extracts of different components of *M. oleifera* and *S. grandiflora* to quench hydroxyl radicals seems to be directly related to the prevention of propagation of the process of lipid peroxidation and seems to be good scavenger of active oxygen species, thus reducing the rate of chain reaction.

H. Antioxidant Activity in Linoleic Acid Emulsion System

The antioxidant effect of two different solvent extract of the *M. oleifera* (OPIF and flower) and *S. grandiflora* (flower and leaf) and α-tocopherol, in preventing the peroxidation of linoleic acid, were measured by thiocyanate method, is shown in Fig. 6. Oxidation of linoleic acid produces hydroperoxides, which decompose to many secondary oxidation products. These oxidized products react with ferrous chloride to form ferric chloride which, on further reaction with ammonium thiocyanate, forms ferric thiocyanate that is red in color. Antioxidants can slow down the peroxidation of linoleic acid, the formation of ferric thiocyanate will be slow. In the present investigation, all the extracts of samples exhibited between 24.67% and 28.18% peroxidation of linoleic acid after incubation for 48 h at 250 µg concentration in the reaction mixture, but it is less efficient compared to the positive control, α-Tocopherol (32.83%). These results suggest that the plant extract might react with free radicals, particularly with peroxy radicals which are the major propagators of the auto oxidation of fat, thereby terminating the chain reaction. Lee and Lim [66] reported the antioxidant activity of ethanol and water extract of ginger was found to be (40.3% and 56.0%). The phenolics compound and other chemical components present in the extract may suppress lipid peroxidation through different chemical mechanisms such as free radical scavenging, electron transfer, radical addition or radical recombination.

TABLE I
RECOVERY PERCENT, TOTAL POLYPHENOLS OF DIFFERENT SOLVENT EXTRACTS OF EDIBLE PARTS OF *M. OLEIFERA* AND *S. GRANDIFLORA* (G 100G^{-1})

Sample	Extract Yield (%)	Total phenolics (mg/g extract)
MPA	19.2	11.86 ± 0.57
MPE	3.2	3.05 ± 0.28
MFA	34.6	10.49 ± 0.43
MFE	4.9	2.49 ± 0.51
SWFA	26.9	2.48 ± 0.16
SWFE	11.8	3.17 ± 0.49
SFLA	14.8	3.31 ± 0.15
SWLE	6.4	3.7 ± 0.44

Values are mean of triplicate determinations (n=3) ± SD.

TABLE II
ABTS $^{+}$ RADICAL CATION SCAVENGING, ANTIHEMOLYTIC AND METAL CHELATING ACTIVITY OF DIFFERENT SOLVENT EXTRACTS OF EDIBLE PARTS OF *M. OLEIFERA* AND *S. GRANDIFLORA*

Sample	TAA* (µmol g^{-1} sample extract)	Antihemolytic activity (%)	Metal chelating activity (mg EDTA/ g sample)
MPA	8563 ± 685.43	63.9 ± 1.9	104.26 ± 1.88
MFA	5930 ± 354.12	68.0 ± 0.2	97.38 ± 2.77
SWFA	2965 ± 279.82	49.3 ± 2.4	57.45 ± 0.94
SWLA	4190 ± 467.19	43.2 ± 0.8	48.84 ± 0.52
MPE	3658 ± 278.48	48.9 ± 1.6	51.55 ± 1.68
MFE	2941 ± 719.05	63.6 ± 0.33	56.90 ± 0.91
SWFE	2135 ± 294.65	60.4 ± 2.1,	49.19 ± 0.42
SWLE	2965 ± 378.72	61.5 ± 0.9	45.16 ± 1.32

Values are mean of triplicate determinations (n = 3) ± SD. * Total antioxidant activity (µmol equivalent Trolox performed by using ABTS radical cation).

IV. CONCLUSIONS

In the present study, the antioxidant activity of aqueous ethanol and acetone extract of *M. oleifera* (OPIF and flower) and *S. grandiflora* (flower and leaf) was evaluated. Overall, it could be concluded that acetone extract of *M. oleifera* (OPIF and flower) and *S. grandiflora* (flower and leaf) bear a potent antioxidant activity. Though the outer pericarp of immature

Review Article *http://www.alliedacademies.org/nutrition-human-health/*

Total phenolic content of organic and conventional green leafy vegetables.

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Abstract

Green Leafy Vegetables (GLVs) contain an immense variety of bioactive non-nutritive health enhancing factors. GLVs have an abundance of phenolic compounds. The AOA of phenolic compounds is mainly due to the redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers, heavy metal chelators and hydroxyl radical quenchers Total phenoplic content (TPC) of organic and conventional GLVs was determined using Folin-Ciocalteu reagent spectrophotometrically with Gallic acid as the standard. Fresh samples of GLVs were extracted separately with water, methanol and ethanol for the estimations. Curry leaves *(Murraya koenigii)*, a commonly used green in most India preparations had the highest phenolic content ranging from 3468.80 ± 88.03 to 5084.53 ± 123.49 µg of GAE/g of FW in all the solvents of both OG and CV farming system. Agathi *(Sesbania grandiflora)* and fenugreek *(Trigonella foenum graecum)* had more polyphenolics in water extract. These greens are generally cooked in water medium; hence they are a valuable source of phenols in the diet. In our study, a one-way between extracts of solvents ANOVA was conducted to compare the effect of solvents used in the extraction of total phenols in each type of farming methods (OG or CV). The results elucidate that the quantity of total phenolics in GLVs varied among different extracting solvents.

Keywords: Green leafy vegetables, Organic, Conventional, Phenols, Folin-Ciocalteu.

Accepted on January 15th, 2018

Introduction

Phenols are aromatic compounds containing one or several hydroxyl groups directly attached to the benzene ring. According to the number of hydroxyl groups, phenols are classified as dihydric, trihydric and polyhydric. By the year 2005, thousands of polyphenolic compounds had been isolated from plants [1]. There are many spectrophotometric methods for the quantification of phenolic compounds in plant materials. Based on different principles, these methods are used to determine various structural groups present in the phenolic compounds. Spectrophotometric methods enable either the quantification of all extracted phenolics as a group [2-4] or the quantification of specific phenolic substances.

Green leafy vegetables (GLVs) are micronutrient dense nature's gift to mankind that provide more vitamins per mouthful than any other food. They are a rich source of calcium, iron, β-carotene, vitamin C, dietary fibre and many trace minerals. GLV also contain an immense variety of bioactive non-nutritive health enhancing factors such as antioxidants, phytochemicals, essential fatty acids and dietary fibre. There has been a growing recognition of importance of these phyto nutrients in the prevention of non-communicable disease especially in the past two decades. Various studies reported the presence of abundant phenolic compounds some of them namely quercitin, kaempferol and acacetin in GLVs [5]. The commonly consumed greens are coriander *(Coriandrum sativum)*, curry leaves *(Murraya koenigii)*, amaranth, mint *(Mentha spicata)*, fenugreek *(Trigonella foenum graecum)*, drumstick *(Moringa oleifera)* etc. These greens are inexpensive, and it is advisable to include at 50g in one's diet daily. They contain vital nutrient required for growth and maintenance of health.

Polyphenols encompass several classes of weakly acidic chemicals related to, or built upon the phenyl ring. Polyphenols contain one or more phenolic hydroxyl groups directly attached to these carbon-based aromatic phenyl-ring compounds. Reactive Oxygen Species (ROS) easily oxidize these to quinones, a property that helps account for their free radical scavenging capacity. Polyphenols have diverse functions in plants and are a major class of secondary plant metabolites. The major plant Polyphenols by volume is lignin [6]. Plant polyphenols may have beneficial and/or detrimental effects on mammals. After plants die, phenolic compounds can persist for weeks and affect decomposition. They also have an impact on the shelf life of harvested produce [7].

In the present study, Total Phenolic Content (TPC) of the selected samples was estimated by using Folin-Ciocalteu method. The data obtained was consolidated and tabulated for statistical analyses. We hypothesised that there is no difference between the total phenolic content of OG and conventionally grown GLV. In addition, we used three solvents for the extraction of phenols, viz., water, methanol and ethanol and hypothesised that there is no difference in the quantities of phenols in the extracts using the three solvents.

Materials and Methods

Description of Folin-Ciocalteu reagent

The Folin-Ciocalteu (F-C) method of assay is the simplest method available for the measurement of phenolic content in products. This method is a development of Folin-Denis reagent used in the early 19th century for the determination of tyrosine in proteins [8]. F-C reagent can be prepared by dissolving 100 g sodium tungstate (VI) dihydrate and 25 g sodium molybdate

Table 1. Total phenolic content of organic green leafy vegetables.

S. no	Name of the Green Leafy Vegetable	(µg of GAE/ g of FW)			
		Ethanol	Methanol	Water	Total
1	Agathi *(Sesbania grandiflora)*	1455.9 ±49.33	1473.85 ±37.02	1661.65 ±36.56	4591.50[a]
2	Arakeerai *(Amaranthus tristis)*	Samples not available			
3	Coriander *(Coriandrum sativum)*	404.28 ±20.47	477.62 ±39.25	349.16 ±30.51	1231.06[f]
4	Curry *(Murraya koenigii)*	4926.76 ±259.54	5084.53 ±123.49	4357.54 ±155.52	14368.83[a]
5	Moringa *(Moringa oleifera)*	3414.63 ±113.26	3222.07 ±66.43	2606.72 ±48.54	9243.42
6	Fenugreek *(Trigonella foenum Graecum)*	1210.43 ±33.47	1420.38 ±28.11	1599.63 ±58.68	4230.44[d]
7	Pulicha *(Hibiscus canabinus)*	3544.34 ±166.57	3769.17 ±228.95	177.23 ±5.47	7490.74
8	Manathakkali *(Solanum nigrum)*	931.95 ±27.47	1235.26 ±37.37	1051.96 ±10.17	3219.17[e]
9	Mint *(Mentha spicata)*	1123.32 ± 79.27	2294.72 ±112.01	1151.08± 47.96	4569.12[c]
10	Ponnaganni *(Alternathera sessilis)*	1214.56 ±33.63	1190.96 ±49.10	875.57 ±10.31	3210.09

Table 2. Total phenolic content of conventional green leafy vegetables.

S. no	Name of the Green Leafy Vegetable	(µg of GAE/ g of FW)			
		Ethanol	Methanol	Water	Total
1	Agathi *(Sesbania grandiflora)*	1310.57 ±55.36	1247.32 ±44.82	1382.59 ±34.29	3940.48[i]
2	Arakeerai *(Amaranthus tristis)*	376.83 ±28.99	379.99 ±10.97	451.98 ±18.23	1208.8
3	Coriander *(Coriandrum sativum)*	767.93 ±37.55	894.74 ±28.45	626.88 ±8.89	229.55[f]
4	Curry *(Murraya koenigii)*	3468.8 ±88.03	4171.32 ±112.89	4404.1 ±506.91	12044.22[a]
5	Moringa *(Moringa oleifera)*	Samples not available			
6	Fenugreek *(Trigonella foenum Graecum)*	562.24 ±13.52	576.23 ±34.29	744.8 ±37.30	1883.27[d]
7	Pulicha *(Hibiscus canabinus)*	Samples not available			
8	Manathakkali *(Solanum nigrum)*	886.86 ±27.52	1160.65 ±27.70	983.53 ±40.56	3031.04[e]
9	Mint *(Mentha spicata)*	1116.09 ±31.04	2046.25 ±86.74	1096.92 ±138.72	4258.26[c]
10	Ponnaganni *(Alternathera sessilis)*	Samples not available			

The other GLVs were found to have varying levels of phenols. The quantity of phenolics ranging from 177.23 ± 5.47 to 5084.53 ± 123.49 expressed as µg of GAE/g FW of GLVs. In the OG and CV GLVs methanolic extracts had the highest TPC in mint *(Mentha spicata)*, coriander *(Coriandrum sativum)* leaves, manathakkali leaves *(Solanum nigrum)*. Curry leaves *(Murraya koenigii)* had higher phenolic content in OG methanolic extracts whereas agathi *(Sesbania grandiflora)* and fenugreek *(Trigonella foenum graecum)* had more polyphenolics in water extract. The amount of total phenolics in water extracts ranged from 349.16 ± 30.51 to 4404.10 ± 506.91 µg of GAE/g FW.

TPC in curry leaves *(Murraya koenigii)* and fenugreek leaves *(Trigonella foenum graecum)* had similar amount of Polyphenols [15] which was 158.33 mg of tannic acid/ 100 g. However, some others [16] reported that total phenols in fenugreek leaves *(Trigonella foenum graecum)* was 217.5 mg of catechol/ 100 g FW. Reported TPC of some common GLVs ranged from 5.0 to 69.5 mg of TAE/ g of extracts [17]. Therefore, it can be said that the TPC of vegetables varies widely depending on the variety of vegetables. Comparison is difficult, as method of extraction solvents used; method adopted to extract phenol and standard compounds adopted varied among the studies.

Curry leaves *(Murraya koenigii)* had the highest extraction yield (1.65%) and TPC was 38.6% among the tested samples of curry leaves *(Murraya koenigii)* [18]. TPC of curry leaves *(Murraya koenigii)* in three different climates (India, Nicaragua and Niger) ranged from 2940-4250 mg of GA/ g of DW [19], in water extracts, it was 257-3234 mg TAE/ 100 g of DW [20]. TPC in absolute methanolic and 70% ethanolic extract was 26.46 ± 0.20 and 12.31 ± 0.18 mg GAE/ g DW respectively [21].

Coriander leaves *(Coriandrum sativum)*, curry leaves *(Murraya koenigii)*, manathakkali leaves *(Solanum nigrum)* and mint

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RESEARCH ARTICLE

Phytochemical Screening and Antioxidant Activity of *Sesbania grandiflora* Leaves Extracts

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ABSTRACT

Sesbania grandiflora, family : Fabaceae commonly known as 'sesbania', is widely used as Indian folk medicine. *S.grandiflora* has the common names of Agati, Corkwood Tree and West Indian Pea. In India it is known as vaka or basna. Traditionally *Sesbania gradiflora* is used alone or with other medicinal plants to treat a variety of ailments. The plant's extracts were screened for the presence of phytochemicals and were used to determine their free radical scavenging activity. The extracts showed potent antioxidant activity in the scavenging of DPPH radicals. The potent antioxidant activity of the ethanolic and aqueous extracts of *Sesbania gradiflora* may be due to the high phenolic content such as tannins and flavonoids, was proved by estimation of total phenolic content.

KEYWORDS: *Sesbania grandiflora*, Phytochemical Screening, Phenolic content, Antioxidant activity

1. INTRODUCTION:

Cells under aerobic conditions are threatened to the insult of reactive oxygen metabolites (ROMs) that are efficiently taken care by the powerful antioxidant system in the human body. The term antioxidant can be defined as any substance that delays or inhibits oxidative damage to a target molecule. Antioxidant enzymes, together with the substances that are capable of either reducing ROMs or preventing their formation, forms buffers which have strong reducing properties which effects the capability of the cell to counteract the activation of ROM. All reducing agents thereby form protective mechanisms, which maintain the lowest possible levels of ROMs inside the cell. The first line of defense against O_2.- and H_2O_2 mediated injury are antioxidant enzymes like SOD and CAT[1]. Reactive oxygen species (ROS), such as superoxide anions, hydrogen peroxide, and hydroxyl, nitric oxide and peroxynitrite radicals, play an important role in oxidative stress related to the pathogenesis of various important diseases[2,3].

In healthy individuals, the production of free radicals is balanced by the antioxidative defense system; however, oxidative stress is generated when equilibrium favors free radical generation as a result of a depletion of antioxidant levels. Cancer chemoprevention by using antioxidant approaches has been suggested to offer a good potential in providing important fundamental benefits to public health, and is now considered by many clinicians and researchers as a key strategy for inhibiting, delaying, or even reversal of the process of carcinogenesis[4,5]. Moreover, knowledge and application of such potential antioxidant activities in reducing oxidative stresses *in vivo* has prompted many investigators to search for potent and cost-effective antioxidants from various plant sources. These research activities have contributed to new or renewed public interests worldwide in herbal medicines, health foods, and nutritional supplements. Botanicals have been used for treatment or prevention of various human diseases throughout history. The cancer chemopreventive activities of naturally occurring phytocompounds is of great interest. Many indigenous herbal plants of regional interest have been used popularly as folk medicines in Asian; however, their bioactivities or pharmacological effects are remained to be elucidated.

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Table4. Determination of total polyphenol content of leaves extract of *S.grandiflora*

Extracts	Total polyphenol content (GAE mg/gm)
Ethanolic extract	88.56±0.09
Aqueous extract	90.12±0.52

Data expressed as gallic acid equivalent (GAE) mg/gm of the extract,
Values are mean ± SEM of triplicate determinations

4. DISCUSSION AND CONCLUSION:

In the present study, certain phytoconstituents such tannins, saponins, carbohydrates, steroids, flavonoids and phenolic compounds were detected during qualitative phytochemical screening of the successive leaves extracts of *Sesbania grandiflora*.

Recently herbal products and synthetic drugs as antioxidant agents have received much attention. Many plant extracts and herbal products have been reported to possess significant antioxidant activity[17,18]. *Sesbania grandiflora* selected for the present study possess several ethnomedical uses related to its antioxidant activity. Plants containing important phytoconstituents like phenolic compounds, tannins, flavonoids and terpenoids are known to possess antioxidant properties.
Free radicals are implicated in various pathological conditions like tissue injury, inflammatory conditions, neurodegenerative diseases, cancer and aging. The ability of Free radical scavenging compounds to ameliorate diseased conditions is appreciated. Thus the human body is protected by antioxidants against damage by reactive oxygen species[19].

The proton radical scavenging action is known to be one of the important mechanisms for measuring antioxidant activity. This assay determines the scavenging of stable radical species DPPH by antioxidants compounds present in the extracts. The rates of DPPH scavenging activity of extracts are probably due to the presence phenolic compounds. Our study clearly indicated that the extracts exhibited high content of phenolic compounds which was significantly correlated with the DPPH radical scavenging activity.

Now it may be concluded that among the two leaves extracts like ethanolic and aqueous of *Sesbania grandiflora* tested for its *in vitro* antioxidant activity, both the extracts exhibited potent antioxidant activity with significant IC_{50} values in the scavenging of DPPH radicals. The potent antioxidant activity of the ethanolic and aqueous extracts may be due to the high phenolic content such as tannins and flavonoids.

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INVESTIGATION OF PHYTOCHEMICALS AND ANTIOXIDANT POTENTIAL OF LEAF EXTRACT OF *Sesbania grandiflora* L. Pers

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ABSTRACT

Nowadays there is resurgence of interest in the potential health benefits of natural remedies like medicinal plants and their extracts. The presence of antioxidants such as phenolics and flavonoids in plants may provide protection against a number of diseases and have attracted much interest because of their ability to scavenge free radicals. Medicinal plants are therefore investigated for their antioxidant properties, and their demand is increasing worldwide. The present study was aimed to screen the phytochemicals as well as to evaluate the antioxidant potential of ethanolic extract of *Sesbania grandiflora* L. Pers leaves. Finely powdered leaves of *S.grandiflora* (white variety) were macerated using 95% ethanol. The ethanol extract was subjected to phytochemical screening and estimation of total phenol and flavonoid contents. *In vitro* antioxidant assays such as DPPH, Nitric oxide and Hydrogen peroxide radical scavenging assays were performed to determine the free radical scavenging activity. Qualitative phytochemical screening revealed the presence of alkaloids, flavonoids, terpenoids, phenolics, tannins, steroids, anthraquinone glycosides, carbohydrates and proteins. Total flavonoid and phenol contents in 10 mg of extract were found to be 0.956 mg and 0.136 mg of standard quercetin and gallic acid respectively. The extract showed satisfactory scavenging in all the antioxidant assays with potent activity against hydrogen peroxide radical with an IC_{50} of 39.25 µg /ml. Antioxidant activity of the ethanol extract of *S.grandiflora* can be attributed to the presence of flavonoid and phenolic contents.

KEYWORDS: Free radicals, Reactive oxygen species, Phenolics, Flavonoids.

axis and concentration on the X axis. Linear regression analysis were used to determine the concentration of EESG from the calibration curve The absorbance values obtained for different concentrations of standard are tabulated in table 4.

Table 4: Mean absorbance of different concentrations of the standard gallic acid.

Concentration of gallic acid (µg/ml)	Absorbance (750nm)
100	0.1801 ± 0.001
200	0.2712 ± 0.003
400	0.3500 ± 0.001
800	0.6806 ± 0.002
1000	0.8500 ± 0.001

Absorbance expressed as Mean ± SD, n=3

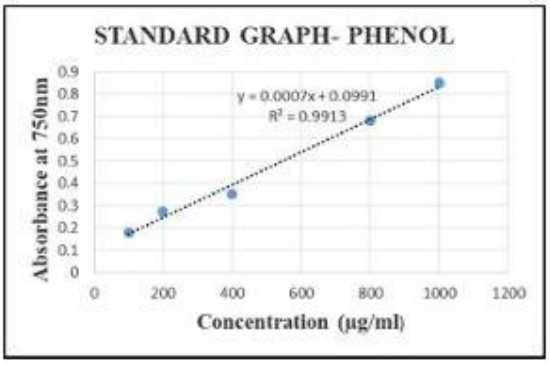

Fig 2: Calibration curve of gallic acid.

Table 5: Estimation of total phenolic content of EESG.

Absorbance of EESG(nm)	Gram equivalent of gallic acid per 10mg
0.0910	0.137mg

The total phenolic content in 10 mg of EESG was found to be equivalent to 0.137 mg of standard gallic acid.

- **Antioxidant Assays**

1. DPPH radical scavenging assay: The absorbance of different concentrations (6.25-100µg/ml) of standard (ascorbic acid) were measured at 517 nm and the percentage inhibition obtained were tabulated on table 6. A graph of concentration Vs percentage inhibition of DPPH free radical by EESG and standard ascorbic acid was plotted and shown in the fig 1.

Qualitative phytocompounds were screened in *S.grandiflora* leaves, according to the results alkaloids, flavonoids, terpenoids, phenolics, tannins, steroids, anthraquinone glycosides, carbohydrates and proteins were present.

As the results of preliminary phytochemical screening shown the presence of phenols and flavonoids, total flavonoid and phenolic content estimation was also performed in ethanolic extract of *S.grandiflora* leaves .The total flavonoid content of EESG was determined by using Aluminium chloride colorimetric method. The total flavonoid content in 10mg of EESG was found to be equivalent to 0.9560 mg of standard quercetin. The total phenolic content in EESG was determined by Folin Cio-calteau method and 10mg of EESG was found to be equivalent to 0.1365 mg of standard gallic acid. Phenolics are secondary plant metabolites which possess a wide range of therapeutic uses such as antioxidant, anti-mutagenic, anti-carcinogenic and free radical scavenging activities while flavonoids are a group of phenolic compounds which exhibit several biological effects such as anti-inflammatory, anti-hepatotoxic, antiulcer, anti-allergic, anti-viral, free radical scavenging and anticancer activities.

In this study, free radical scavenging activity of EESG was determined by various *in vitro* assay models such as DPPH free radical, NO free radical and H_20_2 free radical scavenging assays. Free radicals are unstable chemical species that cause damage to lipid cells, proteins and DNA as a result of imbalance between the generation of reactive oxygen species (ROS) and the antioxidant enzyme. Some examples of these free radicals are superoxide anions, hydroxyl, nitric oxide and hydrogen peroxide radicals. These free radicals may oxidize nucleic acids, proteins, lipids and DNA and can initiate many neuro degenerative diseases Antioxidants are compounds that are capable of either delaying or inhibiting the oxidation process which occurs under the influence of atmospheric oxygen / reactive oxygen species DPPH assay is widely used as a preliminary test that provides information on the reactivity of test compound with a stable free radical since odd electron of DPPH gives strong absorption band at 517nm and when it is quenched by the extract, there is a decrease in absorbance. EESG showed anti-radical activity in scavenging DPPH radical with a maximum inhibition of about 60.58 % at a concentration of 100 µg/ml. IC_{50} values of standard ascorbic acid was found to be 9.72 µg/ml while that of EESG was found to be 48.88µg/ml.

NO is a free radical with a single unpaired electron. NO itself is not a reactive free radical, however overproduction of NO is involved in ischaemic perfusion, neurodegenerative and