

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^{·+} 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 (43.2 % 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 hydroxytoluene (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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Moringa oleifera Lamark is the most widely cultivated species of monogeneric family, the *Moringaceae*, as an important medicinal plant, which is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan [15], [16]. About 10 to 12 species of *Moringa* are considered to be native to India, from here they have been introduced in many warm countries. It is a small, fast, growing, evergreen or deciduous and drought tolerant tree that usually grows up to 10 or 12 m in height even in poor soil conditions also. It yields high biomass and adapt to unfavorable environmental conditions [17]. Flowers are white, scented in large axillary down penicles, pods are pendulous, ribbed, and seeds are 3- angled. *Moringa* is categorized as a leafy vegetable like the leaves of the baobab, manioc, sweet potato, amaranth and hibiscus. Various parts of these plants such as leaves, flowers and pods are having excellent nutritive supplements with various pharmacological properties [18], [19]. Leaves, seeds, fruits, root and flower extract of *M. oleifera* are sources of biologically important phytochemicals and nutraceutical molecules including glucosinolates and phenolic compounds [20], gallic acid, chlorogenic acid, ellagic acid, ferulic acid, kaemferol and quercetin [21], [22] water soluble polysaccharide [23] and nitril glycosides (niaziridine and niazirin) [24], volatile compounds [25], and aminoacids. The uses of *Moringa* species are diverse such as roots, leaves, flowers, green pods and seeds are used for the human foods and stems and petioles are used as forage materials [21], [26]. Seeds are utilized for the production of cooking oil [26], biodiesel [27], and source of water purification agent [28] and mosquito repellent [29]. Medicinally leaves, roots, barks, seeds and flowers of *Moringa* are generally known for their multiple pharmacological effects including antimicrobial activity [30], immune mediated inflammatory responses [31], anticancer [32], anti hyperglycemic [18], [33], [34], hepatoprotective [35], hypocholesterolemic [36], and wound healing properties [37].

Leafy vegetables inclusion in diet has been shown to be protective against various chronic and age-related disorders due to the presence of antioxidants. Knowledge of its nutritional and antioxidant properties will promote the utilization of these underutilized leafy vegetables in all parts of the country. This study was therefore, set out to evaluate the antioxidant nutrient properties of the leaves and white flower of *Sesbania grandiflora* and flower and outer pericarp of immature edible fruit of *Moringa oleifera* investigating the free radical scavenging activities and determining its total polyphenolic contents.

II. MATERIALS AND METHODS

A. Chemicals

Potassium ferricyanide, trichloroacetic acid, linoleic acid, 2, 2'-diphenyl-1-picrylhydrazyl (DPPH), 2, 2- azinobis (3-ethylbenzo-thiozoline-6-sulfonic acid) disodium salt (ABTS), 6-hydroxy -2,5,7,8-tetra-methylchroman 2- carboxylic acid (Trolox), ferrous chloride, ammonium thiocyanate, hydrogen peroxide, ferrous ammonium sulfate, ethylene diamine tetra

acetic acid (EDTA) disodium salt, ascorbic acid were obtained from Hi Media, Merck or Sigma. All other reagents used were of analytical grade.

B. Samples and Processing

Fresh flowers and immature fruits (edible stage) of *Moringa oleifera* (Lam), the leaves and white flowers of *Sesbania grandiflora* (L.) Pers (white variety) were collected from Pollachi, Coimbatore district of Tamil Nadu. Soon after collection, they were shadow dried at room temperature ($27 \pm 2^\circ\text{C}$) for 5-9 days. Then the dried samples were fine powdered and stored in a separate screw capped bottles until further analysis. The samples were labeled as follows, MPA- *M. oleifera* Pericarp Acetone extract; MPE- *M. oleifera* Pericarp Ethanol extract; MFA- *M. oleifera* Fruit Acetone extract; MFE- *M. oleifera* Fruit Ethanol extract; SWFA- *S. grandiflora* White Flower Acetone extract; SWFE- *S. grandiflora* White Flower Ethanol extract; SWLA- *S. grandiflora* White Leaf Acetone extract; SWLE- *S. grandiflora* White Leaf Ethanol extract.

C. Solvent Extraction

10 gm of each sample weighed and added 50 ml of petroleum ether. After 3 hours for occasionally shaking, the sample was centrifuged at 5000 rpm for 20 min. after centrifugation the supernatant was discarded and pellets were allowed for air-drying. Then 100 ml of 70 % acetone was added with air-dried residues and using orbital shaker, the sample was shaken for 1 hour at room temperature. Then the sample was centrifuged at 5000 rpm for 20 mins and the supernatant was saved and the pellets were re-extracted twice with each 50 ml of 70% acetone for 4 hours. After centrifugation the combined supernatant were allowed for air drying and further the extract was kept in an oven at 40°C for removal of residual solvent and moisture and to attain constant weight. The remaining air-dried residues (pellets) were extracted with 100 ml of 50% ethanol using the orbital shaker at room temperature for one day. Then the contents were centrifuged at 5000 rpm for 20 min and the supernatant was collected in a separate container. The left out residue was re-extracted with 50 ml of 50% ethanol using shaker for 4 hours at room temperature. Then the sample was centrifuged at 5000 rpm and the supernatant was combined with previous extract and air-dried. In order to remove the residual solvent and moisture, the extract was kept in an oven at 40°C for 48 hours. After drying the respective extracts under incubator temperature at 40°C (once after reaching the sample to a constant weight) the recovery percent was calculated as follows:

$$\% = \frac{\text{Extract} + \text{Container (g)} - \text{Empty container (g)}}{\text{Sample (g)}} \times 100$$

Finally both the aqueous acetone and ethanol extracts of respective samples were stored in a separate screw capped vials at room temperature for further chemical analyses.

D. Estimation of Total Phenolic Contents

The total phenolic contents of the aqueous acetone and ethanol extracts of various samples was determined according to Folin-Ciocalteu method (FCM) described by Siddhuraju

and Becker [21]. FCM actually measures a sample's reducing capacity and can be considered as another antioxidant (electron transfer) capacity assay. For the assay, aliquots (100 μ l) of extracts were taken in a test tube and the volume was made up to 1 ml with distilled water. Then 0.5 ml of Folin-Ciocalteu phenol reagent (1:1 with water) and 2.5 ml of sodium carbonate solution (20 %) were added sequentially in each tube. Soon after vortexing the reaction mixture, the test tubes were placed in dark for 40 min and the absorbance was recorded at 725 nm using a UV- visible spectrophotometer (Shimadzu) against the reagent blank. The analysis was performed in triplicate and the results were expressed as the tannic acid equivalents (TAE).

E. Determination of Reducing Power

The reducing power of different solvent extracts was determined according to the method of Oyaizu [38] as described by Yen et al. [39]. 20-100 μ g of extracts was dissolved in 1 ml of methanol was mixed with a phosphate buffer (5 mL, 0.2 M, pH 6.6) and potassium ferricyanide (5.0 mL, 1.0%); the mixture was incubated at 50°C for 20 min. A portion (5.0 mL) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 650g for 10 min. the upper layer of the solution (5.0 mL) was mixed with distilled water (5.0 mL) and ferric chloride (1.0 mL, 0.1%), and then the absorbance was read spectrophotometrically at 700 nm. A higher absorbance of the reaction mixture indicated greater reducing power.

F. Free Radical Scavenging Activity on DPPH

The antioxidant activity of the extracts was determined in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH, according to the method of Blais [40]. Extracts at various concentrations was taken and the volume was adjusted to 100 μ l with methanol. 5 mL of a 0.1 mM methanolic solution of DPPH was added and shaken vigorously. The tubes were allowed to stand for 20 min at 27°C. The absorbance of the sample was measured at 517 nm. Radical scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the following formula

$$\% \text{ radical scavenging activity} = (\text{Control OD} - \text{Sample OD} / \text{Control OD}) \times 100$$

G. Total Antioxidant Activity Assay by Radical Cation (ABTS^{•+})

ABTS was dissolved in water to a 7 mM concentration, ABTS radical cation (ABTS^{•+}) was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at temperature for 12-16 h before use. Prior to assay, the solution was diluted in ethanol (about 1:89 v/v) and equilibrated to 30 °C to give an absorbance at 734 nm of 0.700 \pm 0.02 in a 1cm cuvette. The stock solution of the sample extracts in ethanol were diluted such that, after introduction of a 10 μ l aliquot of each dilution into the assay, they produced between 20-80 % inhibition of the blank absorbance. After the addition of 1 ml of diluted ABTS solution to 10 μ l of samples

or Trolox standards (final concentration 0-15 μ M) in ethanol OD (optical density) was taken at 30°C exactly 30 min after the initial mixing. Appropriate solvent blanks were also run in each assay. Triplicate determinations were made at each dilution of standard, and the percentage inhibition of the blank absorbance at 734 nm was plotted as a function of Trolox concentration [41] described by Siddhuraju and Becker [21]. The unit of total antioxidant activity (TAA) is defined as the concentration of Trolox having equivalent antioxidant activity expressed as μ mol/g sample extracts using the calibration curve of Trolox. Linearity range of the calibration curve was 0.25-1.25 mm/l. The total antioxidant activity of ASC and BHA were also measured by ABTS method for comparison.

H. Antihemolytic Activity

Antihemolytic activity of the extracts was assessed as described by Naim et al. [42]. The erythrocytes from cow blood were separated by centrifugation and washed with phosphate buffer (pH 7.4). The erythrocytes were then diluted with phosphate buffered saline to give 4% suspension. 500 μ g of extract/mL of saline buffer were added to 2 ml of the erythrocyte suspension and the volume was made up to 5 ml with saline buffer. The mixture was incubated for 5 min at room temperature and then 0.5 ml of H₂O₂ solution in saline buffer was added to induce the oxidative degradation of the membrane lipids. The concentration of H₂O₂ in the reaction mixture was adjusted to bring about 90% hemolysis of blood cells after 240 min incubation and then the reaction mixture was centrifuged at 1500 rpm for 10 min and the extend of hemolysis was determined by measuring the absorbance at 540 nm corresponding to hemoglobin liberation.

I. Hydroxyl Radical Scavenging Activity

The scavenging activity of the extracts of raw and processed flower samples on hydroxyl radical were measured according to the method of Klein et al [43]. Extracts were mixed with 1 ml of iron-EDTA solution (0.13 % ferrous ammonium sulphate and 0.26 % EDTA), 0.5 ml of EDTA solution (0.018 %) and 1.0 ml of Dimethyl sulfoxide solution (0.85 % v/v in 0.1 M phosphate buffer, pH 7.4). The reaction was initiated by the addition of 0.5 ml of ascorbic acid (0.22 %) and incubated at 80-90°C for 15 min in a water bath. After incubation the reaction was terminated by the addition of 1.0 ml of ice-cold TCA (17.5 % w/v). 3 ml of Nash reagent was added and left at room temperature for 15 min. The reaction mixture without sample was used as control. The intensity of the color formed was measured spectrophotometrically at 412 nm against reagent blank. The % hydroxyl radical scavenging activity was calculated by the following equation;

$$(\%) \text{ HRSA} = 1 - (\text{Difference in absorbance of sample} / \text{Difference in absorbance of control}) \times 100$$

J. Antioxidant Activity in Linoleic Acid Emulsion System

The antioxidant activity of the extract was determined using the thiocyanate method Kikizaki and Nakatani, [44]. Each sample (250 μ g) in 0.5 ml of absolute ethanol was mixed with 0.5 ml of 2.51% linoleic acid in absolute ethanol, 1 ml of

0.05M phosphate buffer (pH 7.0), and 0.5 ml of distilled water and placed in a screw capped tube. The reaction mixture was incubated in dark at 40°C using an incubator. Aliquots of 0.1 ml were taken at every 12 h during incubation and the degree of oxidation was measured by sequentially adding ethanol (9.7 ml, 75%), ammonium thiocyanate (0.1 ml, 30%) and ferrous chloride (0.1 ml, 0.02 M in 3.5% HCl). After the mixture was rested for 3 min, the peroxide value was determined by monitoring absorbance at 500 nm until the absorbance of the control reached the maximum. The antioxidant activity was calculated as percentage of inhibition relative to the control.

$$AA = 100 - \frac{(\text{Sample absorbance at 48h} - \text{sample absorbance at 0 h})}{(\text{Control absorbance at 48h} - \text{control absorbance at 0 h})} \times 100$$

K. Metal Chelating Activity

Chelating property of the two different solvent extracts of *S. grandiflora* and *M. oleifera*, was assessed by bipyridyl assay [45]. The reaction mixture contained 0.25 ml of 1mM FeSO₄ solution, 0.25 ml of extract, 1 ml of 0.2 M Tris-HCl buffer (pH 7.4), 1 ml of 2, 2' bipyridyl solution, 0.4 ml of 10% hydroxylamine-HCl and 2.5 ml of ethanol. The final volume was made up to 5 ml with deionized water and the absorbance was determined at 522 nm. The chelating activity of the extracts was evaluated using EDTA as standard. The results were expressed as mg EDTA equivalent/g sample extracts.

L. Statistical Analysis

The data were subjected to one-way analysis of variance (ANOVA), and the significance of the difference between means were determined by Duncan's multiple-range test ($p < 0.05$) using SPSS (Version 13.0, SPSS Inc., Wacker Drive, Chicago, USA). Values expressed are means of triplicate determinations \pm standard deviation.

III. RESULTS AND DISCUSSION

A. Recovery Percent and Phenolic Content of Extracts

The yield percent and total phenolic content of extracts obtained from *S. grandiflora* (white variety- flower and leaf) and *M. oleifera* (flower and outer pericarp of edible fruit) using 50% ethanol and 70% acetone solvents are shown in Table I. The maximum yield was recorded in acetone extracts of both plant samples than the ethanolic extracts. Phenolic compounds exist widely in plants which are essential for human to modulate lipid peroxidation implicated in atherogenesis, thrombosis and carcinogenesis owing to their antioxidant and anti-inflammatory activity [21]. Phenolics are important mainly because of their function to scavenge the free radicals in the human body and to help maintaining healthy body by scavenging or removing the reactive oxygen. So, it is sensible to estimate the phenolics and tannins in selected plant extracts. Thus the contents of total phenolics of *M. oleifera* (2.49- 11.86 mg TAE/g extract) and *S. grandiflora* (2.48- 3.7mg TAE/g extract) were recorded and the results are shown in Table I. The present study revealed higher values of phenolics in acetone extract of flower, and outer pericarp of immature fruit of *M. oleifera* than the ethanol extract. Similar

results was accounted by Siddhuraju and Becker [21] who proposed the antioxidant activity of methanolic leaf extract of *M. oleifera* from three different agro-climatic origins in the range of (2.94 and 4.25 g/100 g). On the other hand total phenolics in ethanol extract of flower and leaf of *S. grandiflora* were shown higher than the acetone extracts. The observed result might be because of different degree of polarity of the solvents used for the extraction of phenolics compounds and thus could contribute significantly to the antioxidant and free radical scavenging activity [46]. Shyamala et al. [47] reported the total phenolics content of some common Indian green leafy vegetables was found to be in the range of 5-65.5 mg TAE/ g of extracts.

B. Reducing Power Assay

The reducing power of bioactive compounds was associated with antioxidant activity. Thus it is necessary to determine the reducing power of phenolics constituents to elucidate the relationship between their antioxidant effect and their reducing power [48]. Figs. 1 and 2 shows the reducing power of the extracts using potassium ferricyanide reduction method. The reducing power of the extracts increased with an increase in concentration; It has been reported that the reducing power was associated with the antioxidant activity and its relationship of phenolic constituents has been well established in several plant sources including vegetables [21], [49]. Yellow color of the test solution changes to various shades of green and blue depending upon the reducing power of each extract. The presents of reductant in the herbal extracts causes the reduction of Fe³⁺/ Ferric cyanide complex to ferrous form. Therefore Fe²⁺ complex can be monitored by measuring the formation of Perl's Prussian blue at 700nm [38]. Aqueous acetone extract of all the samples of both plants shows higher reducing power and the values were comparable to that of tannic acid. The reducing properties of ferric ion are often used as an indicator of electron donating activity and can reduce the oxidized intermediates of lipid peroxidation processes, which is an important mechanism of phenolics antioxidant activity. Radical chain reactions would terminated by high reducing tendencies of substances which convert the free radicals into more stable products. Similar observation between the polyphenolic constituents in terms of dose dependent and reducing power activity have been reported for different Moringa leaf [21], Moringa pod [50], arial root of *Ficus racemosa* and *F. bengalensis* [51], different cultivars of banana flower [52], and fruit and flower extract of *Hypericum lydiium* [53].

C. DPPH Radical Scavenging Activity

DPPH radical scavenging method has been commonly used to evaluate the hydrogen donor or free radical scavenging ability of plant extracts [54]. The presence of antioxidant activity in the crude extract of two different solvent of various edible parts of *Moringa oleifera* and *Sesbania grandiflora* with DPPH decolorized the visible purple color by measuring the changes in absorbance at 517 nm. The degree of

discoloration based on the scavenging potential of the plant extract because of its hydrogen donating ability.

In this study, both solvent extracts scavenged DPPH radicals in a concentration dependent manner, activity was increased with increasing concentration, while highest activity was observed in the acetone extract of both plant samples. The results are expressed as a percentage manner and shown in Figs. 3 and 4. At a dosage ranging from 0.2 to 0.6 mg of acetone extract of *M. oleifera* (OPIF and flower) and *S. grandiflora* (flower and leaf) shows higher percentage of free radical scavenging activity (8.45%-65.03%), whereas at a dose ranging from 2.13 to 6.39 mg of ethanol extracts of *M. oleifera* (OPIF and flower) and *S. grandiflora* (flower and leaf) show low percentage of scavenging activity (4.7%-30.03%) under the experimental conditions, respectively. The acetone extract could serve as the inhibitors of free radicals were able to boost the natural antioxidant defense mechanism. In the present study, the order of sample extract activity is MPA > MFA > SWLA > SWFA > SWLE > MFE > MPE > SWFE. This radical scavenging activity of extracts might be related to the nature of phenolics, thus contributing to their electron transfer/hydrogen donating ability. Variations in the radical scavenging activity of different extracts may be attributed to differences in their chemical composition. It is clearly shown that all the extracts possess the free radical scavenging potential with an increase in the dose of extract. Similar observation was reported for the free radical scavenging activity was found to be increase with increasing concentration of vegetable extracts of *Centella asiatica* (30.98–91.27%), *Murraya koenigii* (7.52-23.89%) at concentration of 0.4–2.0 mg/ ml extract [55], leaf samples of *Cassia tora* Linn aqueous extracts (10.15-77.78%) and acetone extracts (2.10-60.61%) at concentration of 0.01-10.0 mg/ ml extract [56].

D. ABTS^{•+} Radical Cation Scavenging Activity

ABTS^{•+} assay is based on the antioxidant ability to react with ABTS radical cation generated in the assay system. ABTS^{•+} is a method based on reduction of the 2, 2'-azinobis (3-ethylbenzothiazoline sulphonate) radical. ABTS^{•+} have been widely used to measure the antioxidant capacities of natural extract based on their ability to reduce the radical cation, reactions of ABTS^{•+} with free radical scavengers present in the plant sample occur rapidly and can be assessed by following the decrease in the sample absorbance at 734 nm [57]. The effect of acetone and methanol extract of *M. oleifera* (OPIF and flower) and *S. grandiflora* (flower and leaf) on ABTS radical cation scavenging activity is presented in Table II.

In ABTS^{•+} radical cation scavenging assay, the activity of the tested sample extracts was expressed as Trolox equivalent. Even though the samples exhibited good ABTS radical scavenging activity, the 70 % acetone extract of *M. oleifera* (OPIF and flower) showed the highest TAA (8563 and 5930 $\mu\text{mol g}^{-1}$, respectively) than the ethanolic extract (3658 and 2941 $\mu\text{mol g}^{-1}$, respectively) and also acetone extract of *S. grandiflora* (leaf and flower) showed the highest TAA (4190

and 2965 $\mu\text{mol g}^{-1}$, respectively) than the ethanolic extract (2965 and 2135 $\mu\text{mol g}^{-1}$, respectively). The acetone extract had strong activity to quench ABTS radical due to the presence of phenolics compounds with hydroxyl group attached to the aromatic ring structures [58]. It was considered that the acetone extract of *M. oleifera* (OPIF and flower) and *S. grandiflora* (leaf and flower) was a good source of potent natural antioxidant activity. Its high TAA value denoted that the mechanism of antioxidant action of this acetone extract was a hydrogen donor and it would terminate the oxidation process by converting free radicals to the stable forms.

E. Antihemolytic Activity

To elucidate the biological relevance of the antioxidant activity of acetone and ethanolic extract of *M. oleifera* (OPIF and flower) and *S. grandiflora* (flower and leaf), the cow blood erythrocytes was used herein as cell-based model system. The H₂O₂- induced oxidative damage on cow erythrocytes has been extensively studied as model for the peroxidative injury in biological membranes [42] and also it's useful for screening studies on various molecules and their metabolites having antioxidant activity [59]. H₂O₂ generates peroxy radicals that attack the erythrocytes to induce the chain oxidation of lipids and proteins, disturbing the membrane organization and eventually leading to hemolysis. In this study, the protective effect of the acetone and ethanolic extract of *M. oleifera* (OPIF and flower) and *S. grandiflora* (flower and leaf) on hemolysis by peroxy radical scavenging activity was investigated.

All the extracts, showed antihemolytic activity in terms of percentage inhibiting activity ranging from (43.2 % to 68.0 %) (Table II). Antihemolytic activity of extracts of *M. oleifera* (OPIF and flower) and *S. grandiflora* (flower and leaf) were found that did not show any harmful effects to erythrocytes. Polyphenols are well-known effective scavengers of free radicals. Therefore, phenolics compounds of various components of acetone and ethanolic extract of *M. oleifera* (OPIF and flower) and *S. grandiflora* (flower and leaf) present in the incubation medium most likely quench the peroxy radicals formed in the aqueous phase before these radicals attack the biomolecules of the erythrocyte membrane cause oxidative hemolysis. Similarly, a highly significant efficiency in inhibiting radical induced red blood cell hemolysis was also observed for *Camellia sinensis*, *Ficus bengalensis* and *F. racemosa* [51], leaves of *Laser trilobum* [60]. The data obtained from this antihemolytic activity of the extracts revealed that it could be free radical inhibitors and behaves as primary antioxidants which react with free radicals.

F. Metal Chelating Activity

An important function of polyphenolic antioxidants is the terminators of free radicals and chelators of metal ions that are capable of catalyzing lipid peroxidation. The ability of the extracts to chelate transition metals is therefore considered to be due to an antioxidant mechanism. Therefore, it was considered of importance to screen the iron (II) chelating ability to the extracts. The chelation of Fe²⁺ ions by the

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* ($\mu\text{mol g}^{-1}$ 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 ($\mu\text{mol equivalent Trolox performed by using ABTS radical cation}$).

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

fruit of *M. oleifera* is considered to be a waste but it possesses good radical scavenging property. The constituents of crude extracts of *M. oleifera* and *S. grandiflora* which scavenge free radicals and exert a protective effect against oxidative damage induced to cellular macromolecules. The antioxidant potential may be attributed to the presence of polyphenolic compounds and consumption of these traditional leafy vegetables may therefore be a good source of phytochemicals tender varied opportunities for functional food as well as plant-based pharmaceutical products that it offer and make it more useful in dietary constituents of common public in world over.

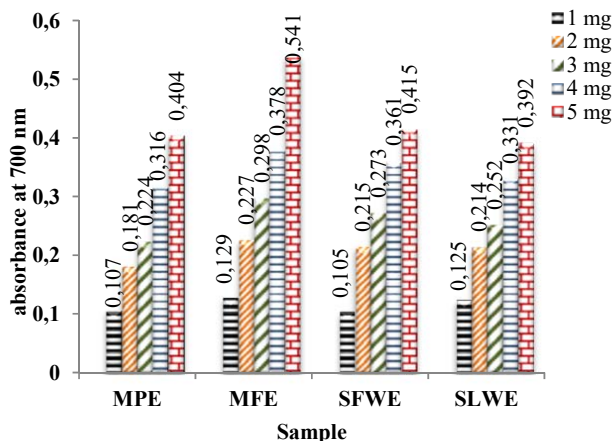


Fig. 1 Reducing power activity of ethanolic extracts of *M. oleifera* (outer pericarp of immature fruit and flower) and of *S. grandiflora* White (flower and leaf) *Values are mean of triplicate determinations (n=3) ± SD

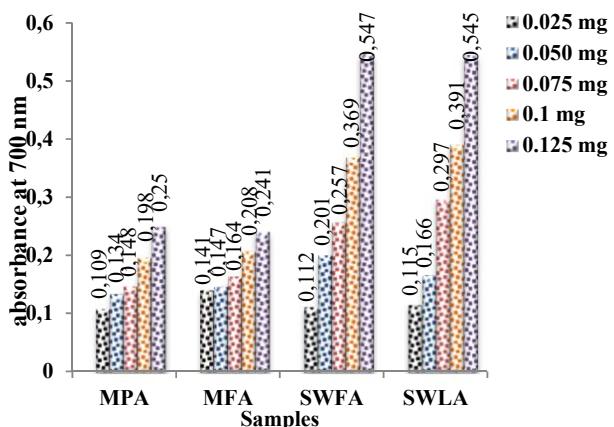


Fig. 2 Reducing power activity of acetone extracts of *M. oleifera* (outer pericarp of immature fruit and flower) and of *S. grandiflora* White (flower and leaf) *Values are mean of triplicate determinations (n = 3) ± SD

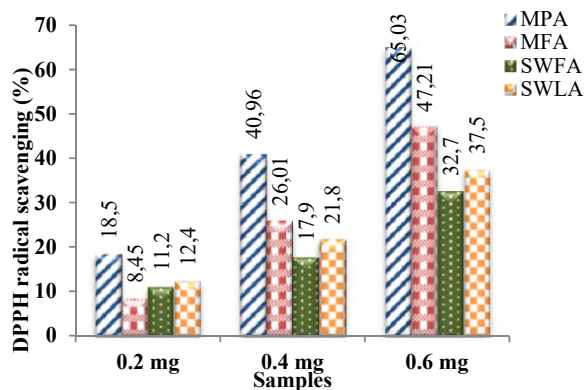


Fig. 3 DPPH radical scavenging activity of acetone extract of *M. oleifera* (outer pericarp of immature fruit and flower) and of *S. grandiflora* White (flower and leaf) *Values are mean of triplicate determinations (n=3) ± SD

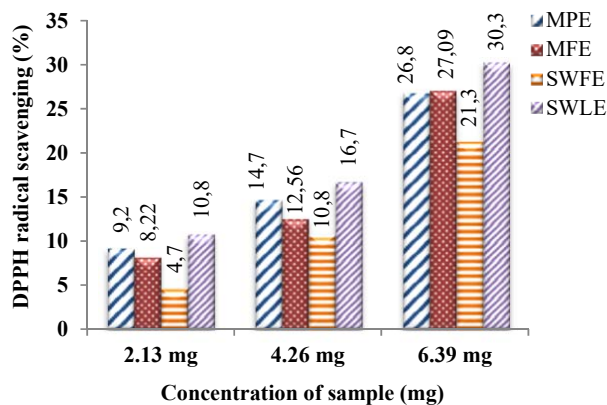


Fig. 4 DPPH radical scavenging activity of ethanolic extract of *M. oleifera* (outer pericarp of immature fruit and flower) and *S. grandiflora* white (flower and leaf) *Values are mean of triplicate determinations (n=3) ± SD

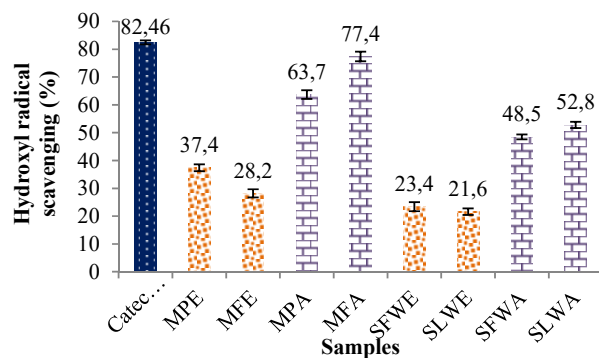


Fig. 5 Hydroxyl radical scavenging activity of acetone and ethanol extract of *M. oleifera* (outer pericarp of immature fruit and flower) and *S. grandiflora* White (flower and leaf) *Values are mean of triplicate determinations (n =3) ± SD

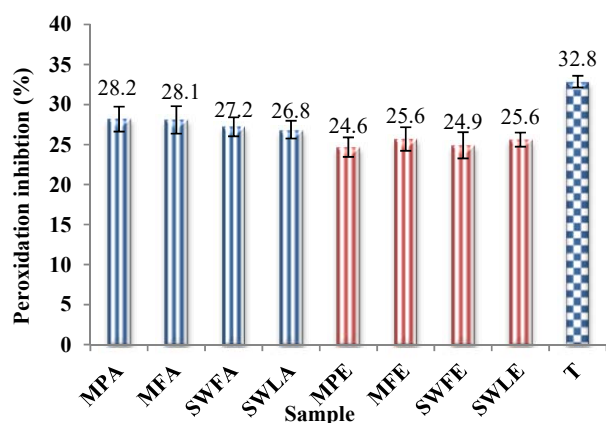


Fig. 6 Peroxidation inhibition property of acetone and ethanol extract of *M. oleifera* (outer pericarp of immature fruit and flower) and *S. grandiflora* White (flower and leaf) at a concentration of 250 μ g in the reaction mixture *Values are means of triplicate determinations (n=3) \pm standard deviation. T- α -tocopherol

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