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Effect of geographical properties on the phytochemical composition and antioxidant potential of *Moringa oleifera* flowers

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ABSTRACT

Moringa oleifera is a major tropical plant with highly valued nutritional and medicinal properties. The present study is undertaken to analyze the phytochemistry and antioxidant activity of the flowers of this plant and study the influence of the geographical properties on them. Antioxidant activities of extracts of *M. oleifera* flowers from four different districts of Tamilnadu, India with varying geographical properties, were investigated. Free radical scavenging potential was evaluated in vitro by using diphenyl-picryl-hydrazyl (DPPH) assay. Other antioxidant assays like Hydroxyl radical scavenging assay, Superoxide Anion Radical scavenging assay, Metal chelating assay, Phosphomolybdenum assay, Ferric thiocyanate (FTC) method, Thiobarbituric acid (TBA) method were also adopted. Phytochemicals in the flower extracts were screened qualitatively. Phenol and flavonoid contents of the samples were estimated. Significant differences were observed in the antioxidant machinery of the extracts. Though the presence of phytochemicals among the samples remained the same, their quantity varied as noticed from their phenol and flavonoid contents which correlated to their antioxidant activities. The methanol extract of *M. oleifera* flowers from Tirunelveli district with loamy red soil, moderate monsoon and less pollution, exhibited the highest antioxidant potential. Its phenol and flavonoid contents also seemed to be maximum when compared with the other samples. Results suggested the profound effect of geographical properties on the phytochemical composition which further influenced the antioxidant potential of *M. oleifera* flowers.

Keywords: Antioxidant potential, Geographical properties, *Moringa oleifera*, Phytochemical composition

INTRODUCTION

Plants with their wide variety of chemical constituents offer a promising source of agents with medicinal properties. Besides forming the backbone of traditional medicine, these plants continue to support human health and welfare. In recent years, considerable attention is directed towards natural food and food ingredients from plants that are believed to be safer and healthier than their synthetic counterparts. It is interesting and worthwhile to investigate such edible plants.

Most of the medicinal applications of plants are established by their antioxidant activities. Antioxidants quench, scavenge and suppress the formation or oppose the actions of free radicals and reactive oxygen species (ROS) [1]. Super oxide radicals, hydroxyl radicals, hydroperoxyl radicals that result from the cellular redox process are generally regarded as ROS. These radicals have been implicated in several major oxidative stress related diseases including heart disease, diabetes and cancer. In treatment of these diseases, antioxidant therapy has gained an immense importance. But, currently available synthetic antioxidants like butylated hydroxy anisole, butylated hydroxyl toluene, tertiary butyl hydroquinone and propyl gallate have been suspected to prompt negative health effects. Hence, their application is strongly restricted and there is a need to substitute them with naturally occurring antioxidants derived from plant source. Among the natural antioxidants, phenols and flavonoids from plants, being electron rich and capable of neutralizing ROS, have appeared as the favored choice.

Moringa oleifera Lam. is a predominant tropical nutritional plant with high medicinal value. It is the most widely cultivated species of the family Moringaceae. It is rich in a number of vitamins, minerals and specific phytochemicals, reported to have hypo-tensive, anticancer, and antibacterial activities [2]. The plant is rich in compounds containing rhamnose, glucosinolates and isothiocyanates [3]. The tree's fruits, flowers, leaves, pods, young shoots and roots are all consumed as food and have an impressive range of medicinal uses. The flowers are used to make tea for colds and improve the quality and flow of breast milk. They possess good amounts of both calcium and potassium. They are cholagogue, stimulant, tonic and diuretic. Folk medicine supports the use of *M. oleifera* flowers for treating cancerous tumors [4]. Several decades of research have been more on the other parts of this medicinal plant. But only limited research is done on the flowers. Therefore, in the current study, focus is laid on the flowers to explore their antioxidant potential and phytochemistry.

Agro-climatic conditions and environment can influence the chemical composition and therapeutic properties of medicinal plant species as recognized and documented in Ayurveda [5]. Earlier findings have suggested that temperature, soil type and other environmental fac-

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tors are responsible for the variations in the chemical constituents and antioxidant potentials of the plants from different geographical locations. *M. oleifera* is widely cultivated throughout the tropical belt [6]. India, a tropical country, is the largest producer of *M. oleifera* with an area of production of 380 km², out of which 74.08 km² is from Tamilnadu. This is the pioneering state in that it has diversified geographical areas with varied genotypes and introductions from Sri Lanka [7]. Thus, the districts of this state with varying geographical properties are selected for the study.

Confirmation of bioconstituents and biological activities of plants across varied agro-ecologies is necessary in the selection and formulation of plant-based food supplement and development of food-based medicinal compounds. The present study is therefore undertaken to investigate for the first time, the effect of geographical properties on the phytochemical composition and antioxidant potential of *M. oleifera* flowers.

MATERIALS AND METHODS

Chemicals

All reagents used for the study were of analytical grade and purchased from Sigma Aldrich, Mumbai and Gemini Scientific Company, Chennai.

Plant material

Fresh flowers of *Moringa oleifera* Lam. were collected during the season February to March from the farms of four different districts of Tamilnadu, India, with varying geographical properties. Chennai in Chennai district (A), Panruti in Cuddalore district (B), Palayamkottai in Tirunelveli district (C) and Kannivadi in Dindigul (D) were the locations used for sample collection. The four samples were identified and authenticated by Dr. Sasikala Ethirajulu, Assistant Director (Pharmacognosy), Siddha Central Research Institute, Chennai. The flowers were cleaned and shade dried for 5 days and the dried material was powdered and subjected to direct extraction.

Plant sample extraction

Using direct method of extraction [8], 10 grams of air dried powder of the flower sample was extracted with 100 ml of the solvent in shaking condition. Initially, sample A from native locality was chosen for extraction with solvents of varying polarities, namely, water, ethanol, methanol, acetone, ethyl acetate and hexane. Later, samples B, C and D were extracted with methanol alone which showed the maximum antioxidant activity among the extraction solvents checked with the sample A. The extract was decanted in to pre-weighed glass vials. The process was repeated 3 times with the same material but using fresh solvent. The solvent was removed by condensation. The extracted residue was measured for its yield and used for further analysis.

Determination of antioxidant activities

1, 1 diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity [9]

DPPH radical was used for the determination of free radical-scavenging activities of aqueous, ethanol, methanol, acetone, ethyl acetate and hexane extracts of sample A to select the best extracting solvent and of methanol extracts of samples A, B, C and D to select the best sample with the highest activity. Different concentrations (20 - 200 µg ml^{-1}) of each extract were added, in equal volume, to the ethanolic solution of DPPH (100 µM). The mixture was left at dark for 20 minutes at room temperature and the absorbance was read at 517 nm.

Hydroxyl radical scavenging assay [10]

Various concentrations (50, 100, 150 and 200 µg ml^{-1}) of methanol extracts of samples A, B, C and D were taken and added to 1 ml of iron– Ethylenediaminetetraacetic acid (EDTA) solution (0.13% ferrous ammonium sulfate and 0.26% EDTA), 0.5 ml of EDTA (0.018%), and 1 ml of dimethyl sulphoxide (DMSO 0.85% v/v in 0.1 M phosphate buffer, pH 7.4). The reaction was initiated by adding 0.5 ml of 0.22% ascorbic acid. Test tubes were capped tightly and heated on a water bath at 80–90 °C for 15 min. The reaction was terminated by the addition of ice-cold trichloroacetic acid (17.5% w/v). Three ml of Nash reagent (75 g of ammonium acetate, 3 ml of glacial acetic acid, and 2 ml of acetyl acetone were mixed and made up to 1 litre with distilled water) was added to all the tubes. The tubes were allowed to stand at room temperature for 15 min. Intensity of the yellow color formed was measured at 412 nm against the reagent blank.

Superoxide radical scavenging assay [11]

The methanol extracts of samples A, B, C and D of concentrations 50, 100, 150 and 200 µg ml^{-1} were added to the reaction mixture consisting of 1 ml of nitro blue tetrazolium (NBT) solution (156 µM NBT in 100 mM phosphate buffer, pH 7.4) and 1 ml NADH solution (468 µM NADH in 100 mM phosphate buffer, pH 7.4). The reaction was started by adding 100 µl of phenazine methosulfate (PMS) solution (60 mM PMS in 10 mM phosphate buffer, pH 7.4) to the mixture. The reaction mixture was incubated at 25 ºC for 5 min and the absorbance was measured at 560 nm against blank.

Metal chelating assay [12]

To the methanol extracts of samples A, B, C and D of varying concentrations 50, 100, 150 and 200 µg ml^{-1}, 0.05 ml of 2 mM Ferrous chloride was added. The reaction was initiated by the addition of 0.2 ml of 5 mM Ferrozine and the mixture was shaken vigorously and kept at room temperature for 10 min. Absorbance was measured at 562 nm.

Phosphomolybdenum assay [13]

An aliquot each of different concentrations (50, 100, 150 and 200 µg ml^{-1}) of methanol extracts of samples A, B, C and D was mixed with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) and incubated in a water bath at 95 ^{0}C for 90 min. The absorbance of the mixture was measured at 695 nm.

Ferric thiocyanate (FTC) method [14]

The methanol extracts of samples A, B, C and D with concentration 100 µg ml^{-1} in 99.5% ethanol were mixed with 4.1 ml of 2.51% linoleic acid in 99.5% ethanol, 8 ml of 0.05 M phosphate buffer at pH 7 and 3.9 ml of distilled water and kept under dark conditions at 40°C. To 0.1 ml of this solution, 9.7 ml of 75% ethanol and 0.1 ml of 30% ammonium thiocyanate was added. After 3 minutes, 0.1 ml of 2 M ferrous chloride in 3.5% hydrochloric acid was added to the reaction mixture. Absorbance was measured at 500 nm every 24 hours until one day after the absorbance of the control reached maximum.

Thiobarbituric acid (TBA) method [15]

The reaction mixture containing 2 ml of 20% trichloroacetic acid, 2 ml

of 0.67% 2-thiobarbituric acid and 1 ml of sample solution (100 µg ml^{-1} methanol extracts of samples A, B, C and D), as prepared in FTC method, was placed in a boiling water bath. After cooling, it was centrifuged at 3000 rpm for 20 min. Absorbance of the supernatant was measured at 552 nm. Antioxidant activity with respect to the control, was based on the absorbance on the final day of FTC method.

Statistical calculation

The experiments were carried out in triplicates. Results were expressed as mean ± SD. The data were analyzed for statistical significance by Analysis of Variance (ANOVA) using the statistical tool, Statistics to use [16]. Data were considered significant at $p < 0.05$. Correlations between antioxidant activities and phenol and flavonoid contents among samples were predicted using Microsoft Office Excel 2007.

Phytochemical analysis

The methanol extracts of the samples were analyzed for their phytochemicals qualitatively using the standard procedures with slight modifications [17] and quantitatively for their phenol and flavonoid contents.

The amount of phenolic compounds in the extracts was determined by the Folin Ciocalteu method and calculated from a calibration curve obtained with the reference compound, Gallic acid as the standard (1 mg ml^{-1}). From the standard solution 0.1 to 0.5 ml was taken. Aliquots of 1 mg ml^{-1} of methanol extracts of the samples were taken in separate test tubes. To all the tubes, 0.5 ml of Folin Ciocalteu reagent was added followed by 2 ml of 2% Sodium carbonate. The contents were mixed and incubated for 15 minutes. The absorbance was measured at 765 nm. The standard curve was prepared by 100, 200, 300, 400 and 500 µg ml^{-1} solutions of Gallic acid.

Aluminium chloride colorimetric method was used for flavonoid determination. Methanol extracts of the samples (10 mg ml^{-1}) each of 0.1ml was separately mixed with 0.1 ml of 10% aluminium chloride methanol solution and 0.1 ml of 1 M potassium acetate. The contents were kept at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 415 nm. A calibration curve was prepared for the standard quercetin solution at concentrations 20 to 140 µg ml^{-1}.

The samples (100µg) were checked for the correlation between their respective phenol, flavonoid contents and the various antioxidant activities predicted.

RESULTS AND DISCUSSION

Yield of *Moringa oleifera* flower extracts

Nature of the extracting solvent is one the important factors that determines the extract yields and the resulting antioxidant activities of the plant materials. These activities differ according to the phytoconstituents of varied chemical characteristics and polarities that may or may not be soluble in the solvent of interest.

The yield of different solvent extracts of the samples as presented in Table 1 indicated the proportion of compounds in them. The percentage of yield of the extracted residue of the sample A was in the order: Aqueous> Methanol> Ethanol> Acetone> Ethyl acetate> Hexane. Though the yield was greater in the aqueous extract, the methanol extract which showed the highest antioxidant activity was chosen for further studies between the samples. Many studies have proved the effective antioxidant ability of methanol extracts over other solvent extracts [18]. Moreover, methanol is extensively employed for the recovery of phenolic compounds from plant matrix. Based on its polarity, methanol can easily release cell wall bound polyphenols and neutralize the activity of polyphenol oxidase that metabolizes the polyphenols in plants.

Among the methanol extracts of the samples studied, the yield was maximum in the sample C, followed by the samples B and D respectively. These values were much greater than those obtained for the various solvent extracts of the sample A. This suggested that the trend of the phytochemical composition of the samples might be C>B>D>A.

Table 1: Percentage yield of *Moringa oleifera* flower extracts

Sample	Extract	Weight of dry powder (g)	Weight of dry extract (g)	Yield (%)
A	Aqueous[a]	10	2.17 ± 0.01**	21.70 ± 0.10**
	Methanol[a]	10	2.13 ± 0.02**	21.30 ± 0.20**
	Ethanol[a]	10	1.86 ± 0.02**	18.60 ± 0.20**
	Acetone[a]	10	1.62 ± 0.02**	16.20 ± 0.20**
	Ethyl acetate[a]	10	1.12 ± 0.01**	11.20 ± 0.10**
	Hexane[a]	10	0.33 ± 0.01**	3.30 ± 0.10**
B	Methanol[b]	10	2.51 ± 0.01**	25.10 ± 0.10**
C		10	2.58 ± 0.02**	25.80 ± 0.20**
D		10	2.30 ± 0.02**	23.00 ± 0.20**

[a] Extracts of varying polarities of Moringa oleifera flowers collected from Chennai (A)

[b] Methanol extracts of Moringa oleifera flowers from different districts of Tamilnadu:

B-Dindigul, C-Tirunelveli, D-Cuddalore.

*Results are mean ± SD of three parallel measurements. Statistically significant at **p<0.01 when compared between different extracts of A and between methanol extracts of A, B, C and D.*

Antioxidant activities

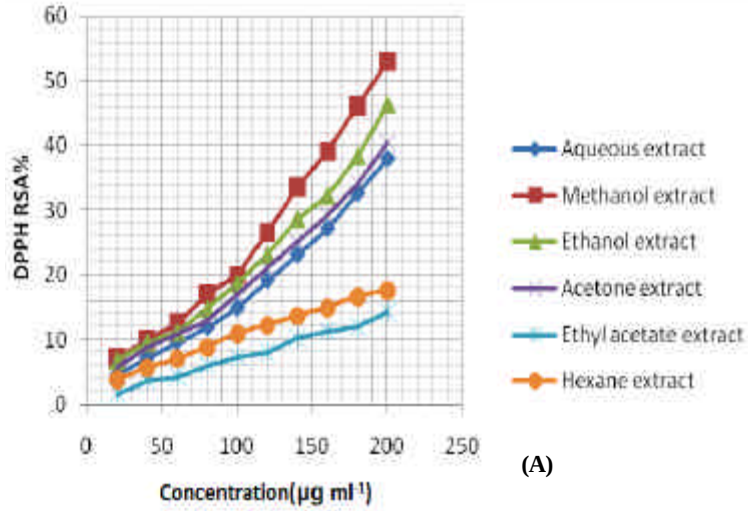
DPPH radical scavenging assay

DPPH is a purple colored stable free radical which turns yellow on reduction by hydrogen donation. Antioxidants react with DPPH and convert it to 1, 1-diphenyl-2-picrylhydrazine, hence considered as radical scavengers. The degree of discoloration indicates the scavenging potential of the antioxidant extract.

DPPH radical-scavenging assay was chosen for its simplicity, rapidity, sensitivity and reproducibility. This method also seemed to be very convenient for the screening of large numbers of samples with different polarities. Results as in Fig. 1A showed that the methanol extract was the most active in the DPPH test among the various solvent extracts of Chennai sample of *M. oleifera* flowers with an IC_{50} (concentration at 50% inhibition) value of 192±0.15 µg ml^{-1}. This was followed by the ethanol, acetone and aqueous extracts whose IC_{50} values were 210±0.26, 230±0.21 and 245±0.10 µg ml^{-1} respectively. The hexane and ethyl acetate extracts showed less activities and their IC_{50} values were above 250 µg ml^{-1}. When considering the methanol extracts of *M. oleifera* flowers from different districts of Tamilnadu as depicted in Fig. 1B, DPPH radical scavenging activity was maximum

for the sample C from Tirunelveli (IC_{50}=125±1.53 µg ml^{-1}) followed by the sample B from Dindigul (IC_{50}=160±1.73 µg ml^{-1}) and the sample D from Cuddalore (IC_{50}=185±0.15 µg ml^{-1}). The sample A from Chennai showed the least activity. The activities of all the extracts increased with rise in sample concentration, though the activities were lower than the standard α-tocopherol (IC_{50}=88±0.06 µg ml^{-1}).

Earlier studies reported 65% of DPPH radical scavenging activity by 200 µg ml^{-1} of methanol extract of *M. oleifera* leaves collected from Neyveli in Cuddalore district of Tamilnadu [19]. The value seemed to be greater than the methanolic flower extract from Panruti in the same district but lesser than that from Palayamkottai in Tirunelveli district which showed the same activity at concentrations of 225 µg ml^{-1} and 165 µg ml^{-1} respectively. Another study on 80% ethanol extract of *M. oleifera* pods from Tonk district, Rajasthan predicted DPPH inhibition of 50.6 ± 0.2% at a concentration of 1000 µg ml^{-1} which was much lesser than that of all the samples A, B, C and D [20].



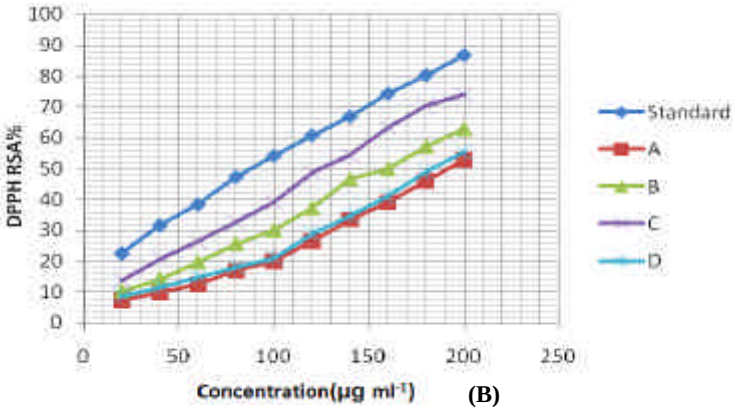


Fig.1. DPPH radical scavenging activity % is expressed for different concentrations of (A) *Moringa oleifera* flower extracts using solvents of varying polarities. Sample: Flowers from Chennai. (B) Methanol extracts of *Moringa oleifera* flowers collected from 4 different districts of Tamilnadu. A-Chennai, B-Dindigul, C-Tirunelveli, D-Cuddalore. Standard: a-tocopherol. Results are mean ± SD of three parallel measurements. Statistically significant at **p<0.01 when compared between different extracts and between different concentrations.

Hydroxyl radical scavenging activity

Hydroxyl radical is found to be an extremely reactive and highly damaging free radical. The radical is capable of combining with DNA nucleotides and abstracting hydrogen atoms from unsaturated fatty acids.

The hydroxyl radical scavenging activities of the methanol extracts of samples A, B, C and D were dose dependent as shown in Fig. 2A. The highest activity among the samples was expressed by the sample C with IC_{50}=237±2.08 µg ml^{-1}. This was followed by the sample B with IC_{50}=272±2.00 µg ml^{-1}. IC_{50} values of the samples D and A were above 300 µg ml^{-1} and of the standard α-tocopherol was 118±0.12 µg ml^{-1}.

Superoxide radical scavenging activity

Superoxide anion is capable of generating singlet oxygen and hydroxyl radicals which may lead to redox imbalance and harmful physiological consequences. The samples showed the scavenging activity in a dose-dependent manner, by donating their electrons to the superoxide and preventing their interaction with NBT. Sample C (IC_{50}=231±0.58 µg ml^{-1}) revealed the maximum scavenging potential among the samples as represented in Fig. 2B. IC_{50} values of the sample B and the standard α-tocopherol were 294±3.63 µg ml^{-1} and 82±2.52 µg ml^{-1} respectively, while for the samples D and A, the values were above 300 µg ml^{-1}.

Metal chelating activity

The transition metal ion Fe^{2+} of iron has been found to cause the production of oxyradicals and lipid peroxidation. Therefore, chelation of these ions affords protection against oxidative damage.

Iron chelating ability of the methanol extract of the samples and the standard EDTA at various concentrations were examined and the values were plotted as in Fig. 2C. Both the sample extracts and EDTA interfered with the formation of ferrous-ferrozine complex suggesting their Fe^{2+} chelating activity to capture ferrous ion before ferrozine. EDTA showed very strong activity (IC_{50} =40±1.53 µg ml^{-1}) while the samples displayed their activities in the order, C>B>D>A with IC_{50} values 128±2.00 µg ml^{-1}, 144±2.52 µg ml^{-1}, 149±2.08 µg ml^{-1} and 168±1.53 µg ml^{-1} respectively. The activities of these samples seemed to be greater than that of 80% ethanol extract of *M. oleifera* pods from Tonk district, Rajasthan which showed an activity of only 41.46 ± 0.02% even at a concentration of 1000 µg ml^{-1} [20].

Total antioxidant activity

Comparison between the samples revealed greater total antioxidant capacity in the sample C with IC_{50} value=272±1.15 µg ml^{-1}. Sample B showed nearer activity with IC_{50} value=278±1.00 µg ml^{-1}. IC_{50} values of the samples D and A were above 300 µg ml^{-1}. The results further indicated that though the antioxidant effect of the samples were lesser than the standard α-tocopherol (IC_{50}=172±0.25 µg ml^{-1}), the antioxidant activity increased with increasing sample concentration as shown in Fig. 2D.

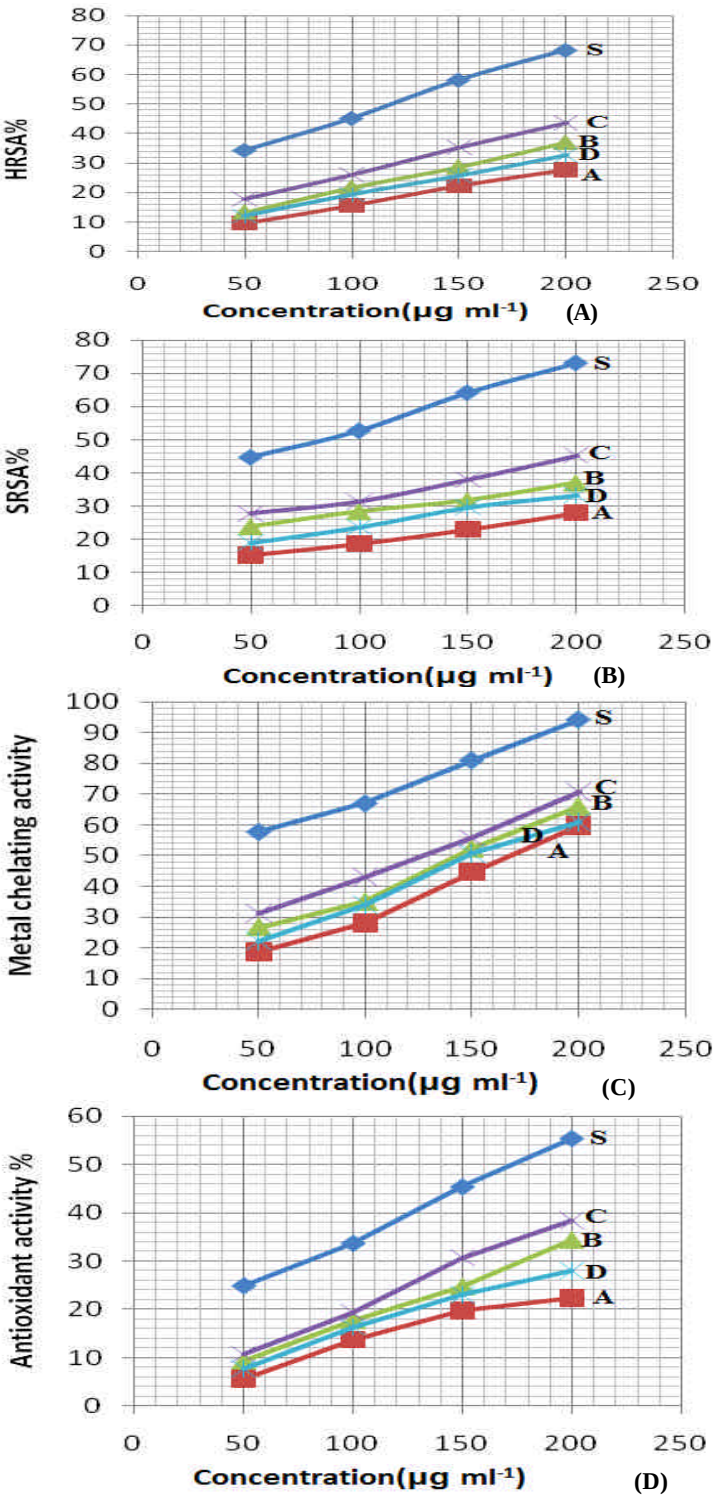


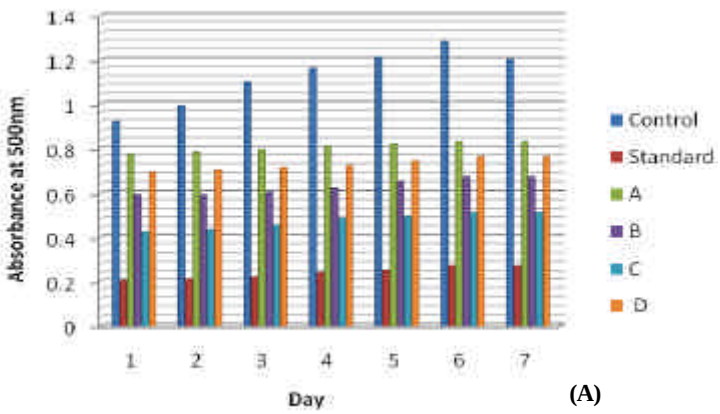
Fig.2. (A) Hydroxyl radical scavenging activities(HRSA%) (B) Superoxide radical scavenging activities(SRSA%) (C) Metal chelating activities(%) (D) Total antioxidant activities(%) of methanol extracts of *Moringa oleifera* flowers. Samples - A, B, C and D. Standard(S): EDTA(Metal chelating activity), a-tocopherol(others). Results are mean ± SD of three parallel measurements. Statistically significant at *p<0.05(Total antioxidant activity) and **p<0.01(others) when compared between different samples and between different concentrations.

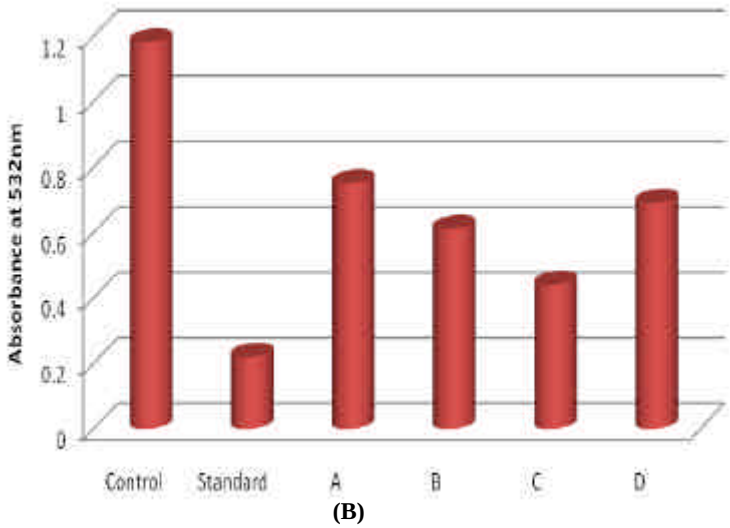
Inhibition of lipid peroxidation

Unsaturated fatty acids in membrane lipids are predominantly susceptible to oxidative processes. Particularly, linoleic acid and arachidonic acid are the usual targets of lipid peroxidation. FTC method is used to measure the peroxide level during the initial stage of lipid oxidation.

Low absorbance values compared to the control indicated high levels of antioxidant activity. Fig. 3A depicted the absorbance values of the methanol extracts of samples A, B, C and D relative to the standard α-tocopherol. The absorbance of the control increased in proportion to the incubation time, and the absorbance of the other samples also increased with increasing incubation time but with a lower increment rate compared to the control. Of the four samples, the increasing order of the antioxidant activity might be given as Sample A (35.16±0.06%) < Sample D (40.63±0.01%) < Sample B (47.66±0.06%) < Sample C (60.16±0.15%) at the concentration of 100 µg ml^{-1} while the standard showed an activity of 78.91±1.00%. The control showed increase in absorbance values from day 1, reached the highest on day 6 and dropped on day 7. This reduction was due to the increased level of malondialdehyde compound from linoleic acid oxidation.

During the oxidation process, peroxides were gradually decomposed to lower molecular weight compounds. One such compound was malondialdehyde, which was measured by the TBA method on the final day of the incubation period, one day after the control reached maximum. Sample C showed the maximum activity of 62.71±0.06% among the samples, followed by the sample B with 48.31±0.06% activity. The activities of the samples D and A were 41.53±0.05% and 36.44±0.04% respectively. The standard showed an activity of 81.36±1.00%. These results correlated well with those obtained previously, using the FTC method. The absorbance values were plotted in Fig. 3B. Sample C showed lower absorbance in both FTC and TBA methods, which indicated that the sample C had higher antioxidant activity when compared to the other samples. Inhibition of lipid peroxidation by these antioxidants might be due to their free radical-scavenging activities. In general, the antioxidant activity by TBA method was higher than that of FTC method. This might suggest the greater stability of the secondary product and thus the amount of peroxide in the secondary stage of lipid per oxidation was more than that in the primary stage.





(B)

Fig.3. Inhibitory effect of methanol extracts of *Moringa oleifera* flowers on lipid peroxidation by (A) FTC method (B) TBA method. Decrease in absorbance with respect to control indicates inhibition of lipid peroxidation. Samples - A, B, C and D. Standard: a-tocopherol. Results are mean ± SD of three parallel measurements. Statistically significant at *p<0.05 when compared between different samples and between different days (FTC method) and at **p<0.01 when compared between different samples (TBA method).

On the whole, the samples exerted a strong inhibitory effect on lipid peroxidation and exhibited greater metal chelating and DPPH radical scavenging activities compared to the other antioxidant activities studied. Dose-dependent variation was observed in the antioxidant activities of all the samples in all the assays and the results were statistically significant between the samples and between the various concentrations.

Phytochemical profile

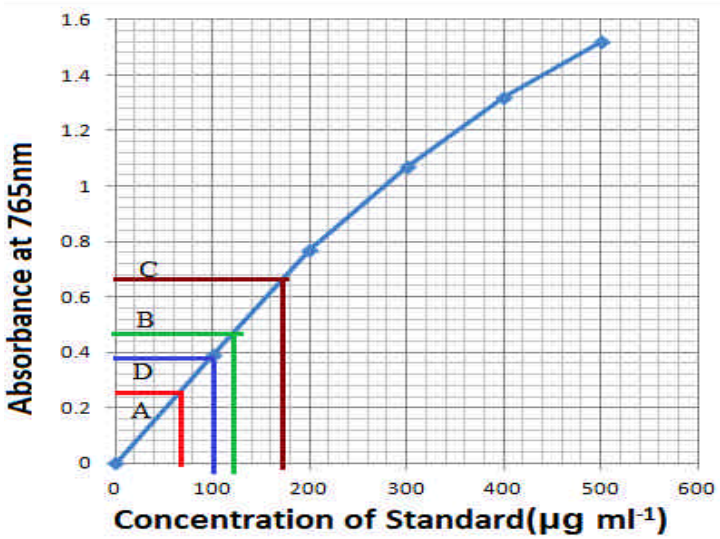
Phytochemicals

Qualitative phytochemical analysis inferred the existence of alkaloids, carbohydrates, glycosides, saponins, proteins, amino acids, phenolic compounds, flavonoids, tannins, terpenoids, steroids, cardiac glycosides, coumarins, quinones, cyanins, fatty acids and organic acids, in the methanol extracts of all the four samples.

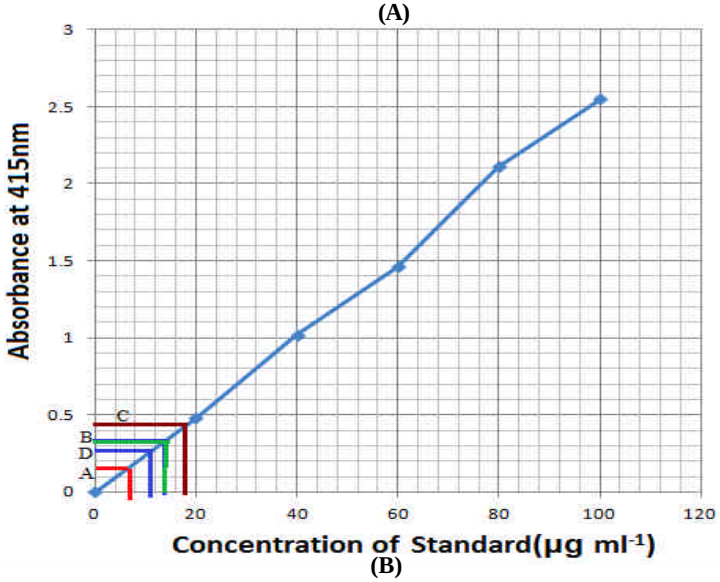
Total phenol and flavonoid content

Absorbance values of the samples at 765 nm were marked on the standard Gallic acid curve as shown in Fig. 4A. The corresponding total phenol contents of the samples were reported as Gallic acid equivalents (GAE) in µg mg^{-1} of dry mass of the sample extract. Sample C showed the highest phenolic content (175.00±8.66 µgGAE mg^{-1}) followed by the samples B(121.00±8.54 µgGAE mg^{-1}), D(100.00±5.00 µgGAE mg^{-1}) and A(68.33±10.41 µgGAE mg^{-1}). Results obtained in an earlier study revealed that the level of phenolic compounds in the methanol extract of *M. oleifera* leaves collected from Neyveli in Cuddalore district of Tamilnadu was found to be 118 ± 3 mg Catechin g^{-1} [19]. The value seemed to be greater than the phenolic content (GAE) of the methanolic flower extract from Panruti in the same district (Sample D) and from Chennai (Sample A) but lesser than that from Dindigul (Sample B) and Tirunelveli (Sample C) districts.

Results for flavonoid content were similar to that of phenol estimation. Absorbance values of the samples at 415nm were marked on the standard quercetin curve as shown in Fig. 4B. The corresponding total flavonoid content of the samples was reported as quercetin equivalents. Sample C showed the highest flavonoid content (18.17±0.76 µg mg^{-1}) followed by the samples B (14.00±1.00 µg mg^{-1}), D (11.50±0.50 µg mg^{-1}) and A (7.00±1.00 µg mg^{-1}).



(A)



(B)

Fig.4. Standard curve for the estimation of (A) Total phenol content (B) Total flavonoid content of methanol extracts of *Moringa oleifera* flowers. Samples - A, B, C and D. Standard: Gallic acid (for phenol content), Quercetin (for flavonoid content). Results are mean ± SD of three parallel measurements. Statistically significant at **p<0.01 when compared between different samples.

An excellent correlation was observed between the total phenol content of the samples and their respective antioxidant activities analyzed earlier. Similar was the correlation between the total flavonoid content and the antioxidant activities. The correlation of the phenol content was found to be greater with the lipid peroxide inhibition by FTC method (R^2=0.991) and with the hydroxyl radical scavenging activity (R^2=0.9957) in case of the flavonoid content as shown in Fig. 5A & Fig. 5B.

A surfeit of phenol and flavonoid compounds from plants had been reported as antioxidant agents, found to play a preventive and therapeutic role in a number of diseases. The strong correlation observed between the antioxidant activities and the phenol and flavonoid contents of the samples suggested a possible use of *M. oleifera* flowers in making the active ingredients of antioxidant supplements. Furthermore, the presence of high content of phenol and flavonoid compounds in *M. oleifera* flowers from Palayamkottai in Tirunelveli district (Sample C) added value to their nutritional and health potential.

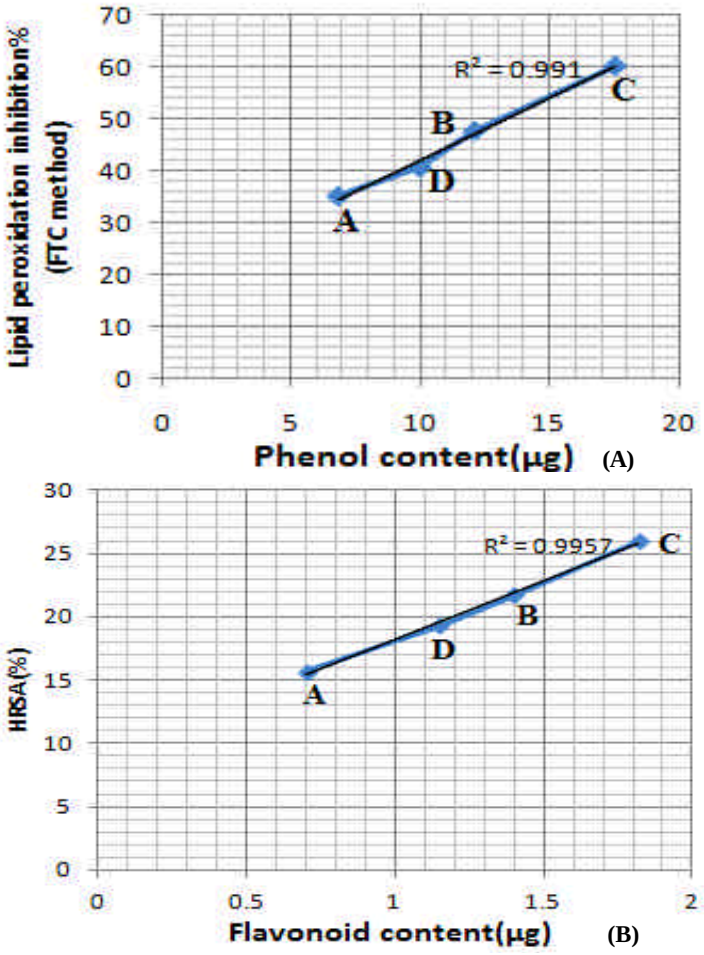


Fig.5. Correlation of (A) Inhibition% of lipid peroxidation (FTC method) with phenol content (B) Hydroxyl radical scavenging activity% (HRSA%) with flavonoid content of methanol extracts(100µg) of *Moringa oleifera* flowers. Samples - A, B, C and D. Correlation coefficient value (R^2) nearest to 1 signifies maximum positive correlation.

Effect of geographical properties

From the results obtained, it was clear that the sample C possessed greater antioxidant potential compared to the other samples studied whose activity decreased in the order B>D>A. On analyzing the factors discriminating these samples of the same species, the major significant barrier seemed to be their geographical properties which affected their growing conditions and hence their phytochemical composition. This was evidenced by the difference in the geographic coordinates of the samples. Sample A was from Chennai (Chennai district) located at 13.08°N 80.27°E, Sample D from Panruti in Cuddalore district at 11.77°N 79.55°E, Sample B from Kannivadi in Dindigul district at 10.38°N 77.83°E and Sample C from Palayamkottai in Tirunelveli district at 8.72°N 77.73°E [21]. This suggested the activity was more towards equator with decrease in the latitude and longitude values. This was in agreement with the previous findings that potent *M. oleifera* grew well in tropics under direct sunlight and that this tree, a strong light-demanding species required 5 by 5 metres spacing for plantations [22]. Though all the samples were in the tropical region, the inclination was more for the sample C followed by the samples B, D and A.

Climatic factors, nature of soil and environment also add on to the observed differences. *M. oleifera* tolerates a wide range of soil conditions, but thrives best in fertile, well drained sandy loams with a neutral to slightly acidic pH of 6.3-7.0. Poor growth is reported on semiarid soils. Minimum annual rainfall requirements are estimated at 250 mm with maximum at over 3,000 mm. Temperature ranges are 25-35 °C, but the tree can tolerate up to 48 °C and can survive a light frost [22].

Tamilnadu with its varied climate has a great variety of soils. The factors like temperature, rainfall, sunlight determine the nature of the soil. While Palayamkottai is a fertile region with loamy red soil and regular monsoon rains coupled with the Thamirabarani river water, Chennai is on the coast of Bay of Bengal with clay, shale and sandstone as the soil and with Adyar and Cooum rivers, heavily polluted with effluents and waste from domestic and commercial sources. Chennai usually relies on the annual monsoon rains to replenish water reservoirs, as no major rivers flow through the area. With a steadily increasing population, the city has faced severe shortage of water and its ground water levels have been depleted. The city also has the problem of excess iron in groundwater [23]. The main rainy season of Palayamkottai is from October to the middle of January due to the southwest monsoon. The average rain fall in the district is 814.8 mm per annum [24]. Chennai gets most of its seasonal rainfall from the north–east monsoon winds, from mid–October to mid–December with an average annual rainfall of about 140 cm. Kannivadi is characterized by semi and tropical monsoon type of climate with the maximum and minimum temperatures recorded as 37.5 °C and 19.7 °C. It is rich in black soil and featured with an average rainfall of 836 mm [25]. Panruti is underlaid with infertile and saline red soil with the normal annual rainfall varying from about 1050 mm to about 1400 mm. The irrigation requirement is usually met by ground water as at times, the deficient monsoon rainfall affects the flow of surface water into reservoir [26].

Supportive references have been gathered from the earlier studies indicating fluctuations in antioxidant activities of plants with growth parameters and environmental factors. One such study has predicted variations in antioxidant attributes at three ripening stages of guava fruit from different geographical regions of Pakistan [27]. Another study, has reported differences in the antioxidant properties of *M. oleifera* leaf extracts from three different agroclimatic origins [28]. The facts from the farmers and official government websites of the districts, along with the literature substantiate the profound effect of agroclimatic and geographic locations on the biological activities of plants. In the present study, the geographical properties of the sample C from Palayamkottai are the best fitting for *M. oleifera* and thus responsible for its highest antioxidant activity among the samples studied.

It was interesting to observe that the phytochemicals in the samples remained the same qualitatively but varied quantitatively as indicated by their phenol and flavonoid contents which were proportional to their antioxidant activities. Thus the sample C with maximum contents of phenols and flavonoids possessed maximum antioxidant activity which in turn was attributed by the geographical properties governing the sample C. Nature of the phytochemicals in the samples did not differ as they belonged to the same species but their quantity varied according to their growing conditions. Previous findings had proposed similar effects. Variabilities were observed in the chemical constituents in the roots of *Coleus forskohlii* from different geographical regions of India [29]. Agroclimatic conditions also influenced the content of cannabinoids in *Cannabis sativa L.* [30]. All these suggested the fundamental role of the geographical properties in differentiating the phytochemical composition of the samples which was further accountable for the observed disparity in their antioxidant potentials.

CONCLUSION

Plants are being used as the richest bio-resource of drugs and food supplements since long. To compete with the growing demand, there is urgency to investigate and scientifically validate medicinally useful plants due to their potent biological activities, low toxicity and economic viability. One such highly valued plant is *Moringa oleifera* with an impressive range of pharmacological and nutritional properties. This study is the first of its kind to evaluate in a comprehensive manner employing a variety of in vitro methods, the antioxidant activities and phytochemical composition of flower extracts of *M. oleifera* from different locations and analyze the effect of geographical properties on them. Successful determination of biologically active compounds from the plant extract is dependent on the type and polarity of the solvent used. Geographical properties, nature and quantity of the bioconstituents play an important role in determining the antioxidant activities of plant extracts. The significant results produced by the methanol extract of *M. oleifera* flowers from Palayamkottai in Tirunelveli district imply their versatility with enormous food and health potentials.

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