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Phytochemical Analysis and Free Radical Scavenging Potential of Herbal and Medicinal Plant Extracts

Anjali Soni* and Sheetal Sosa

ABSTRACT

The objective of the present study was to evaluate the phytochemical constitution and antioxidant activity of methanolic extract of dried leaves of four medicinally important herbs *Ocimum sanctum, Mentha spicata, Trigonella foenum-graecum, Spinacia oleracea* utilized in our routine diet along with one medicinal important tree *Gmelina arborea.* Qualitative analysis of phytochemical constituents' *viz.* tannins, phlobatannins, saponins, flavonoids, steroids, alkaloids, quinones**,** coumarin, terpenoids and cardiac glycosides and quantitative analysis of total phenolics, alkaloids, saponins and flavonoids was performed by the well-known tests protocol available in the literature. Antioxidant activity was studied through DPPH and reducing power assay. The phytochemical screening revealed the extract richness in Tannins, Phlobatannin, Saponins, Flavonoids, Steroids and Alkaloids. Quantitative analysis of phenolics, alkaloids, saponins and flavonoids had revealed that *Mentha spicata* possessed maximum phenolic (18.41 %), *Gmelina arborea* highest alkaloids (5.66 %) & flavonoids (22.80 %) and *Trigonella foenum-graecum* highest saponin (50.12 %) contents. Antioxidant activity was determined by DPPH radical scavenging and reducing power assays. IC_{50} values obtained by DPPH activity for *Mentha spicata* crude extract was found to be 170µg/ml and reducing power was found to be maximum (1.92) at 1mg/ml concentration. The results suggest that *Mentha spicata* has promising antioxidant activity and could serve as potential source of natural antioxidants.

Keywords: Free Radical Scavenging Activity, *Gmelina arborea*, Reactive Oxygen Species, DPPH, Total Phenolic Content, BHT

1. Introduction

Free radicals are chemical species which contains one or more unpaired electrons due to which they are highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability. Free radicals are continuously produced in the human body, as they are essential for energy supply, detoxification, chemical signaling and immune function [1]. Ultraviolet light, ionizing radiation, chemical reactions and metabolic processes can induce the production of reactive oxygen species (ROS) in the cells. Free radicals can initiate the oxidation of bio molecules, such as protein, lipid, amino acids and DNA which will lead to cell injury and can induce numerous diseases [2]. An imbalance between antioxidants and reactive oxygen species results in oxidative stress, leading to cellular damage and oxidative stress is the main cause of several diseases: cancer, cataracts, age related diseases and Parkinson's disease. Antioxidants reduce the oxidative stress in cells and are therefore useful in the treatment of many human diseases, including cancer, cardiovascular diseases and inflammatory diseases. This activity is due to the ability of antioxidants to reduce oxidative stress by neutralizing or scavenging of reactive species by hydrogen donation [3].Medicinal plants contain some organic compounds which produce definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids [4, 5].They are of great importance to the health of individuals and communities. Many of these indigenous medicinal plants are used as spices and food plants. Phenolics have been known to possess a capacity to scavenge free radicals. The antioxidant activity of phenolics is principally due to their redox properties, which allow them to act as reducing agents, hydrogen donors. Phenolics are especially common in leaves, flowering tissues and woody parts, such as stems and barks.

Studies have shown that they play an important preventive role in the development of cancer, heart diseases and ageing related diseases [6].

In the present study the phytochemical screening and antioxidant activities of four medicinally important herbs *Ocimum sanctum* (Tulsi), *Mentha spicata* (Pudina), *Trigonella foenum-graecum* (Fenugreek) and *Spinacia oleracea* (Spinach), used in our routine diet and *Gmelina arborea* (Sewan/ Gamhar). *Gmelina arborea* belonging to family Verbenaceae is a fast growing medicinal tree the leaves of which are used as carminative, in headache, in anasarca, asthma, bronchitis, cholera, colic pain, dropsy, epilepsy, phthisis, rheumatism, small pox, sore, spleen complaints, syphilis, throat swelling, urticaria, as antidote to snake bite and some other poisons, cough, gonorrhea [7]. Though separate studies data on all the herbs taken for our work were available but no reports of systematic studies on comparison of phytochemical content and free radical scavenging activity was reported. These herbs were taken in different ways in our diet as Tulsi and Pudina as one of the tea components, Fenugreek as spice as well as green vegetable, Spinach as vegetable. No reports of studying the antioxidant activity of fast growing *Gmelina arborea* plant were available as per our knowledge of literature studies. *Ocimum sanctum* belongs to the family, Lamiaceae. Tulsi has been widely known for its health promoting and medicinal value for thousands of years. The use of this herb has been reported in Indian Traditional Systems of Medicine and its modern applications are receiving wide spread attention day by day. The plant has also been shown to reduce blood glucose levels, making it an effective treatment for diabetes. *Mentha spicata* is a perennial herb commonly known as mint and spearmint, belong to the family Lamiaceae. *Mentha spicata* have traditionally been used in folk medicine. The distinctive smell and flavor is a characteristic feature of *Mentha* Spp and it is due to the naturally occurring cyclic terpene alcohol called menthol. Menthol is prescribed as a medication for gastrointestinal disorders, common cold and musculoskeletal pain [8]. *Trigonella foenum–graecum* is commonly known as Fenugreek which belongs to the family Fabaceae. *T. graecum* is an important medicinal plant and its leaves and a seed has been used in various ailments and as health tonic. *Spinacia oleracea* is commonly known as "spinach" belonging to family chenopodiaceae. It has been used in the treatment of urinary calculi. It cools and moistens and promotes the urine flow. Beta carotene and lutein in spinach help to maintain healthy eyes and prevent the occurrence of eye disorders like itchy eyes, dry eyes or eye ulcers. It also helps to fight diseases of the bone and skin disorders and helps to protect the health of infants and newborns.

2. Materials and methods

2.1 Collection of Plant Materials

Fresh leaves of selected herbs *Ocimum sanctum, Mentha spicata, Trigonella foenum-graecum, Spinacia oleracea* were purchased from local market and of *Gmelina arborea* from VNSGU campus in the month of February, 2013 and the specimen was authenticated by renowned taxonomist. The leaves were washed, cleaned and chopped into pieces and dried at 40 ^{o}C in thermostatically controlled oven until they attained a constant weight. The samples were then crushed into powder, using mechanical grinding machine, so as to enhance effective contact of solvent with sites on the plant materials.

2.2 Preparation of Plant Extract

10 g of each powdered leaves were placed in conical flask and 100 ml of methanol was added and plugged with cotton. The powder material was extracted with methanol for 24 hours at room temperature with continuous stirring. After 24 hours the supernatant was collected by filtration and the solvent was evaporated to make the crude extract. The residues obtained were stored in airtight bottles in a refrigerator for further use.

2.3 Preliminary Phytochemical Screening

The methanolic extracts of following plants was subjected to different chemical tests for the detection of different phytoconstituents using standard procedures [9, 10, 11].

2.3.1 Test for Tannins: 1 ml of the sample was taken in a test tube and then 1 ml of 0.008 M Potassium ferricyanide was added. 1 ml of 0.02 M Ferric chloride containing 0.1 N HCl was added and observed for blue-black coloration.

2.3.2 Test for Phlobatannins: When crude extract of each plant sample was boiled with 2 % aqueous HCl. The deposition of a red precipitate was taken as evidence for the presence of phlobatannins.

2.3.3 Test for Saponins: Crude extract was mixed with 5 ml of distilled water in a test tube and it was shaken vigorously. Add some drops of olive oil. The formation of stable foam was taken as an indication for the presence of saponins.

2.3.4 Test for Flavonoids: 5 ml of dilute ammonia solution were added to a portion of the crude extract followed by addition of concentrated H_2SO_4. A yellow colouration observed in each extract indicated the presence of flavonoids. The yellow colouration disappeared on standing.

2.3.5 Test for Steroids: 2 ml of acetic anhydride was added to 0.5 ml crude extract of plant sample with 2 ml H_2S0_4. The colour changed from violet to blue or green in samples indicates the presence of steroids.

2.3.6 Test for Alkaloids: Crude extract was mixed with 2 ml of Wagner's reagent. Reddish brown colored precipitate indicates the presence of alkaloids.

2.3.7 Test for Quinones: Dilute NaOH was added to the 1 ml of crude extract. Blue green or red coloration indicates the presence of quinones.

2.3.8 Test for Coumarin: 10 % NaOH was added to the extract and chloroform was added for observation of yellow color, which shows the presence of Coumarin.

2.3.9 Test for Terpenoids (Salkowski test): 5 ml of extract was mixed with 2 ml of chloroform and 3 ml of concentrated H_2S0_4 was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids.

2.3.10 Test for Cardiac glycosides (Keller-Kiliani test): 5 ml of extract was treated with 2 ml of glacial acetic acid containing one

drop of ferric chloride solution. This was underlayed with 1 ml of concentrated H_2SO_4. A brown ring of the interface indicates a deoxy sugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

2.4 Quantitative Determination of Phytochemical Constituents

Determination of TPC

Total phenolic content of the methanolic extract of all selected plants was determined by standard method [12] with little modifications, using tannic acid as a standard phenolic compound. The extracts were diluted with distilled water to a known concentration in order to obtain the readings within the standard curve range of 0.0 to 600 µg of tannic acid/ml. 250 µl of diluted extract or tannic acid solution was mixed with 1 ml of distilled water in a test tube followed by the addition of 250 µl of Folin - Ciocalteu reagent. The samples were mixed well and then allowed to for 5 min at room temperature in order to allow complete reaction with the Folin-Ciocalteu reagent. Then, 2.5 ml of 7 % sodium carbonate aqueous solution was added and the final volume was made up to 6 ml with distilled water. The absorbance of the resulting blue colour solution was measured at 760 nm using spectrophotometer after incubating the samples for 90 min. All the experiment was conducted in three replicates.

2.4.1 Determination of Alkaloids

5 g of the dried powder of each sample was weighed into a 250 ml beaker and 200 ml of 10 % acetic acid in ethanol was added. The mixture is covered and allowed to stand for 4 hours. This was filtered and the extract was concentrated on a water bath until it reaches to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue was the alkaloid, which was dried, weighed and percentage was calculated [11].

2.4.2 Determination of Saponins

20 g of each sample was placed into a conical flask and 100 ml of 20 % aqueous ethanol was added. The samples were heated over a hot water bath for 4 h with continuous stirring at 55 °C. The mixture was filtered and the residue re-extracted with another 200 ml 20 % ethanol. The combined extracts were reduced to 40 ml over water bath at 90 °C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight and saponin content was calculated as percentage [13].

2.4.3 Determination of Flavonoids

10 g of each plant sample was extracted repeatedly with 100 ml of 80 % aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper No 41. The filtrate was allowed to be evaporated into dryness over a water bath and weighed to a constant weight [14].

2.5 *In vitro* Antioxidant activity

2.5.1. 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

The DPPH radical scavenging method was used to evaluate the antioxidant property. The antioxidant activity was compared with that of the natural antioxidant, ascorbic acid. The concentrations of the plant extracts required to scavenge DPPH showed a dose dependent response. The antioxidant activity of each sample was expressed in terms of IC_{50}, and was calculated from the graph after plotting inhibition percentage against extract concentration DPPH assay was carried out after making some modifications in the standard protocol [15]. 1.5 ml of 0.1 mM DPPH solution was mixed with 1.5 ml of various concentrations (10 to 500 µg/ml) of leaf extract. The mixture was shaken vigorously and incubated at room temperature for 30 min in the dark. The reduction of the DPPH free radical was measured by reading the absorbance at 517 nm by a spectrophotometer. The solution without any extract and with DPPH and methanol was used as control. The experiment was replicated in three independent assays. Ascorbic acid was used as positive controls. Inhibition of DPPH free radical in percentage was calculated by the formula:

$$\text{Inhibition (\%)} = [(A_{control}-A_{test})/A_{control}] \times 100$$

Where $A_{control}$ is the absorbance of the control (L-Ascorbic acid) and A_{test} is the absorbance of reaction mixture samples (in the presence of sample). All tests were run in triplicates (n=3), and average values were calculated.

2.5.2 IC_{50} value

Inhibition Concentration (IC_{50}) parameter was used [16] for the interpretation of the results from DPPH method. The discoloration of sample was plotted against the sample concentration in order to calculate the IC_{50} value. It is defined as the amount of sample necessary to decrease the absorbance of DPPH by 50 %.

2.5.3 Determination of Reducing Property

The reducing power of the plant extracts was determined by a slightly modified method [17]. 1 ml of each plant extract concentration (0.1, 0.5 and 1 mg/ml) was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1 %). The mixtures were then incubated at 50 °C for 20 min. Aliquots (2.5 ml) of trichloroacetic acid (10 %) were added to each mixture, which were then centrifuged for 10 min at 1036 x g. The upper layer of the solutions (2.5 ml) were mixed separately with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1 %), and the absorbance levels were measured at 700 nm using a spectrophotometer. Methanol was used instead of the extract as a control. BHT (Butylated hydroxytoluene) was used as positive control and reducing power was reported as BHT equivalent per 100 gm of dry sample.

3. Results and Discussion

3.1 Qualitative analysis of selected species

The result of the preliminary phytochemical screening was carried out on the methanolic extracts of all the samples and revealed the presence of a wide range of phytoconstituents including alkaloids, glycosides, saponins, flavonoids, tannins, steroids supporting the reason for its wide range of biological activities as showed in table 1. Tannins, Phlobatannins, Saponins, Flavonoids, Steroids and alkaloids were found to be universally present in *Ocimum sanctum* (Tulsi), *Mentha spicata* (Pudina), *Trigonella foenum graecum*

(Fenugreek), *Spinacia oleracea* (Spinach) and *Gmelina arborea* (Sewan/ Gamhar) whereas Quinone was absent in all of them. Coumarin, Terpenoids and Glycosides were found to be altogethere absent in *Ocimum sanctum and Trigonella foenum graecum* while all of them was found in *Mentha spicata. Saponins* and *Steroids* were absent in *Mentha spicata* while terpenoids in *Spinacia oleracea* and *Gmelina arborea*. Coumarin and Glycosides were found to *Spinacia oleracea* and *Gmelina arborea.*

Table 1: Phytochemical analysis of methanolic extracts of selected flora.

Phytochemicals	*Ocimum sanctum*	*Mentha spicata*	*Trigonella foenum-graecum*	*Spinacia oleracea*	*Gmelina arborea*
Tannins	+	+	+	+	+
Phlobatannins	+	+	+	+	+
Saponins	+	-	+	+	+
Flavonoids	+	+	+	+	+
Steroids	+	-	+	+	+
Alkaloids	+	+	+	+	+
Quinone	-	-	-	-	-
Coumarin	-	+	-	+	+
Terpenoids	-	+	-	-	-
Glycosides	-	+	-	+	+

3.2 Quantitative analysis of selected species

Medicinal plants constitute the group of plants mainly used for health care. Use of them as traditional medicine is known since time immemorial. Chemicals present universally in all the plants can be classified as primary and secondary metabolites. Primary metabolites include proteins, amino acids, sugars, purines and pyrimidines of nucleic acids, chlorophylls etc., while secondary phytochemicals as alkaloids to terpenoids and acetogenins to different phenols. These are chemically and taxonomically extremely diverse compounds with obscure function. They are widely used in the human therapy, veterinary, agriculture, scientific research and countless other areas [18]. Knowledge of the chemical constituents of plants is desirable because such information will be value for synthesis of complex chemical substances [19, 20, 21]. During present investigation, the phytochemicals were estimated in 4 common herbs and one medicinal plant species collected from Gujarat region.

Table 2: Total Phenolics, alkaloids, saponins and flavonoids in selected flora

Methanolic extracts	Total Phenolics (%)	Total Alkaloids (%)	Total Saponins (%)	Total Flavonoids (%)
Ocimum sanctum	17.65	0.66	0.50	9.85
Spinacia oleracea	**18.41**	1.24	5.27	16.96
Trigonella foenumgraecum	15.31	3.04	**50.69**	20.53
Mentha spicata	12.14	0.84	-	17.90
Gmelina arborea	16.47	**5.66**	5.73	**22.80**

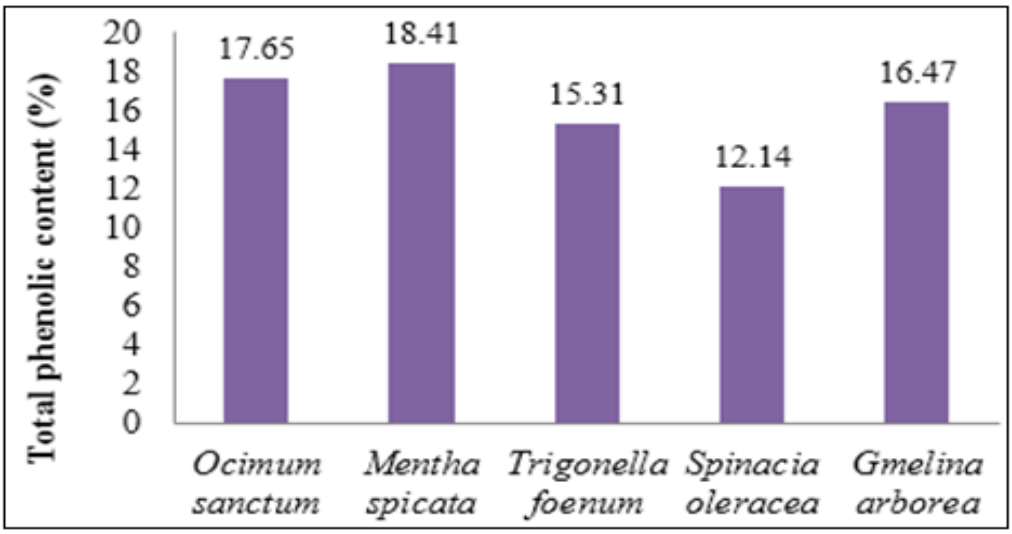

Fig 1: Comparative analysis of total phenolic content in methanolic extracts of selected flora

A comparative study of TPC on all methanolic leaf extract was estimated by using modified Folin-Ciocalteu calorimetric method. Tannic acid was taken as standard and absorbance was recorded at 725 nm. Total phenolic content in selected plants is represented in table 2 and figure 1. Among the samples analysed, *Mentha spicata* was found to contain maximum (18.41 %) and *Spinacia oleracea* contains minimum (12.14 %) phenolics. The total alkaloid content was found to be maximum (5.66 %) in *Gmelina arborea* and minimum (0.66 %) in *Ocimum sanctum*. Total saponin content was maximum (50.69 %) in *Trigonella foenum-graecum* and minimum (0.50 %) in *Ocimum sanctum* while *Mentha spicata* was totally devoid of them. Total flavonoid content was again found to be maximum (22.80 %) in *Gmelina arborea* and minimum (9.85 %) in *Ocimum sanctum.* It can be concluded from present study done on the selected species that *Ocimum sanctum* extract contains mainly phenolics while *Gmelina arborea* contains all phytochemicals in appreciable amounts.

3.3 *In vitro* antioxidant activity

3.3.1 DPPH radical scavenging activity

DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound or plant extracts. DPPH radical scavenging activities of the extracts depended not only on plant type but also upon the extraction solvent. In general, DPPH scavenging activities increased with increasing phenolic components such as flavonoids, phenolic acids, and phenolic diterpenes. These phenolic components possess many hydroxyl groups including o-dihydroxy group which have very strong radical scavenging effect and antioxidant power. In the DPPH assay, the antioxidant was able to reduce the stable radical DPPH to the yellow colored 1, 1-diphenyl-1, 2-picryl hydrazine. The molecule of 1, 1-diphenyl-1, 2-picryl hydrazine is characterized as a stable free radical by virtue of the delocalisation of the spare electron over the molecules a whole. The delocalisation also gives rise to the deepviolet colour, characterized by an absorption band in methanol solution centered at 517 nm. The dose response curve of DPPH radical scavenging activity of crude extracts of plant was observed, when compared with standard ascorbic acid and shown in figure 2. Antioxidant activity in the form of IC_{50} values of different extracts were calculated and shown in table 3. Highest antioxidant activity was given by *Mentha spicata* extract at the concentration of 170µg/ml among all the methanolic leafs which is found to be more than even the ascorbic acid while activity of *Ocimum sanctum* was found to close to the standard. Thus it is clear that polyphenolic antioxidants in leaves of selected plants play an important role as bioactive principles and the scavenging effect can be attributed to the presence of active phytoconstituents in them.

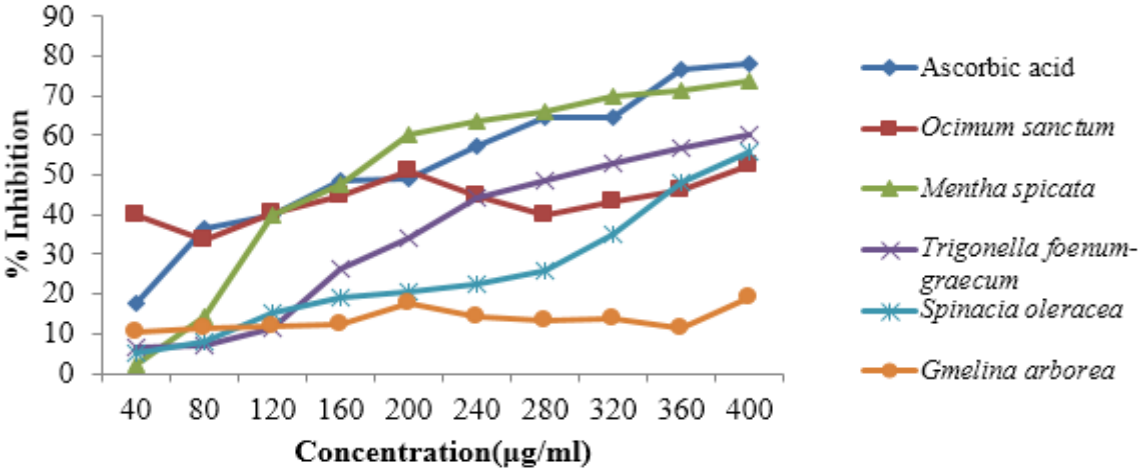

Fig 2: Comparison of Free radical scavenging activity of methanolic plant extrats with standard ascorbic acid

Table 3: Free radical scavenging activity of methanolic plant extracts of selected flora

Sample	IC_{50} (µg/ml)
Ascorbic acid	214
Ocimum sanctum	188
Mentha spicata	170
Trigonella foenum graecum	300
Spinacia oleracea	375
Gmelina arborea	200

3.3.2 Reducing Power assay

Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants. In this assay, the yellow colour of the test solution changes to various shades of green and blue depending on the reducing power of each compound. Presence of reducers causes the conversion of the Fe^{3+}/ferricyanide complex used in this method to ferrous form. By measuring the formation of Pearl's Prussian blue at 700 nm, it is possible to determine the concentration of ferrous ions. Increased absorbance of the reaction mixture indicated increased reducing power of the extracts. Standard curve of BHT was shown in figure 3. The reducing power of all the extracts increased with the increase in their concentration. At 1 mg/ml concentration of the standard BHT the absorbance obtained was 1.57. At the same concentration *Mentha spicata* was found to have the absorbance value 1.92 while all other extracts had low values hence showing that *Mentha spicata* extract possessed very high antioxidant properties.

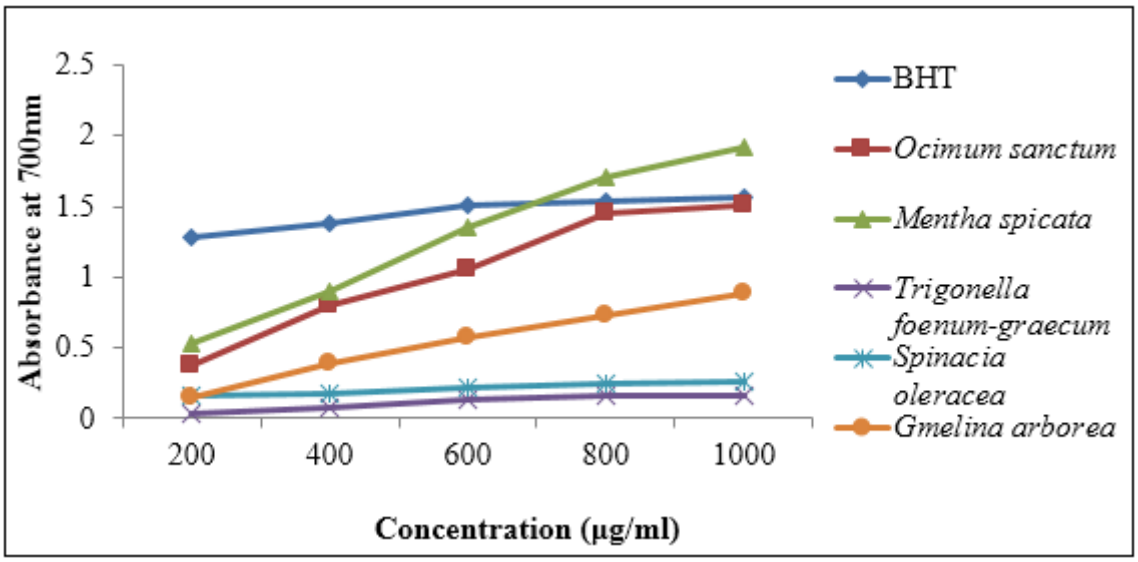

Fig 3: Comparision of reducing power of methanolic extracts of selected flora with standard BHT

4. Discussion

Medicinal value of plants have assumes an important dimension in the past few decades. Plants produce a very diverse group of secondary metabolites with antioxidant potential. Antioxidants block the action of free radicals which have been implicated in the pathogenesis of many diseases and in the aging process [22, 23, 24]. An important role is being played by free radicals in governing the various biological processes which are necessary for the body. They have their role in implicating cell-signaling mechanism occurring in our body. This shows that free radicals are necessary but at the same time harmful for the body. Hence it has a number of mechanisms to minimize free radical induced damage. The damage was repaired with the help of several enzymes like superoxide dismutase, catalase, glutathione, peroxidase and glutathione reductase. In addition antioxidants play a key role in these defense mechanisms which are normally vitamin A, vitamin C, vitamin E and polyphenols [25]. In a study, chemical composition and some anti-oxidant indices of *Alstonia boonei* stem bark extract were evaluated. Presence of alkaloids, tannins, saponins, flavonoids and cardiac glycosides were detected together with important vitamin, ascorbic acid. DPPH radical scavenging activity, total phenolic content and reducing power were 41.58 %, 2.09 mg/g gallic acid equivalent and 0.32 respectively. Results of work had indicated that phytochemicals were responsible for medicinal effects of this plant [26]. In another study, phytochemical constituents and medicinal properties of different extracts of *Anacardium occidentale* and *Psidium guajava* was studied [27]. Aqueous and methanol extracts of leaf, bark, and root cashew and guava were analysed quantitatively for tannin, total polyphenol, oxalate, saponin and alkaloids. Highest concentrations of the bioactive principles were detected in ethanolic extracts of the plants except in the case of saponin where hot water extract produced the bioactive principle. In guava was found tannin-11.5 mg/g, total polyphenol-1.67 mg/g, alkaloid-59.85 % and oxalate-6.66. In cashew tannin-15.38 mg/g, total polyphenolics-2.0 mg/g, alkaloid-39.9 % and oxalate-8.13 % was detected. The study had proved that the presence of these phytochemicals enhances the efficacy and dilutes toxicity also. The total phenolic content of selected Jordanian plant species was done and established that antioxidant activity was closely correlated with phenolic content [28]. During the present work, it was found that *Mentha spicata* exhibited higher antioxidant activity with higher phenolic content. So these findings are in agreement with previous reports that there is linear relation between antioxidant activity and total phenolic contents. Therefore, it can be suggested that the

phenolic compounds significantly contributed to the antioxidant potential of selected plant species. The results are also in agreement with another study done on 112 traditional Chinese medicinal plants [29]. Other group had performed phytochemical analysis of 13 medicinally important plants of Margalla hills and surroundings [30] and investigated the qualitative and quantitative analysis of the major bioactive constituents. Alkaloids, saponins, tannins, anthraquinones, flavonoids, flavons, flavonols and chalcones, terpenoids, phlobatanins, coumarins, steroids and cardiac glycosoides were analyzed qualitatively whereas alkaloids, flavonoids, tannins, phenols and saponins were analysed quantitatively too. Quantitative analysis of total polyphenols, tannins, proanthocyanidins and flavonoids in 20 Serbian and Chinese cultivars of *Soybean* (*Glycine max* L.) was performed [31]. Phytochemical and nutrient evaluation of *Spondias mombin* leaves was performed and reported the qualitative and quantitative analysis of various groups of chemical constituents, minerals and vitamins [32]. The results obtained in this study thus suggest the identified phytochemical compounds may be the bioactive constituents and these plants are proving to be an increasingly valuable reservoir of bioactive compounds of substantial medicinal merit. Quantitative phytochemical estimation and antioxidant studies on aerial parts of *Naravelia zeylanica* DC was done [33]. It was a woody climber belonging to Ranunculaceae family and whole plant is used as medicine for different problems. Powdered plant material was found to have alkaloid 0.86%w/w, total phenol 0.72 %w/w, tannin 8.72 %w/w, flavonoids 0.56 %w/w and saponin 2.86 %w/w were present in the aerial parts. High concentration of phenols and tannins in this plant cause greater reducing powered which in turn responsible due to the presence of these constituents. Quantitative estimation of methanolic extract of various phytoconstituents *viz* total tannins (156.5 mg/g), total phenolics (146.40 mg/g), total flavonoids (30 mg/g) and total flavonols (3.6 mg/g) content on *Cinnamomum wightii* Meissn flowers of family Lauraceae which may serve as diagnostic tools for identification of crude drug [34]. Qualitative and quantitative analysis on plants like *Gymnema sylvestre*, *Tinospora cordifolia*, *Lawsonia inermis*, *Azadirachta indica* and *Ocimum sanctum* having same ayurvedic properties [35]. Phytochemical studies on methanolic and ethyl acetate extracts of leaves of *Anogeissus leiocarpus* and showed that the plant contains alkaloids (152.0 ± 0.1 mg/g), phenolics (1294.81 ± 3.0 mg/g), flavonoids (330.7 ± 3.0 mg/g) in the methanol extra ct and alkaloids (80.20 ± 0.0 mg/g), phenolics (616.5 ± 4.4 mg/g), flavonoids (202.5 ± 4 .0 mg/g) in the ethyl acetate extract respectively [36].The methanol extract of the leaves of the plant *Leucas aspera* has been tested for the determination of antioxidant activity by reducing assay and found that reducing power increases with the increase in concentration of the crude extract [37]. Free radical scavenging potential of the different extracts of leaves of *Oroxylum indicum* (L.) Vent. (Bignoniaceae), one of the widely used medicinal plant, was evaluated *in vitro* by using diphenyl-picryl-hydrazyl (DPPH) assay. The results were expressed as IC_{50}. Ascorbic acid was used as standard showed an IC_{50} of 24.0 ug/mL, whereas, the crude ethyl acetate (I), methanolic (II) and water (III) extracts of leaves of *O. indicum* showed IC_{50} values of 49.0, 55.0 and 42.5 respectively at 100 mg/mL concentration [38].Antioxidant activity evaluation of 28 Chinese herbs was performed by DPPH and reducing power assays in which *Artemisia vulgaris* and *Sanguisorba officinalis* showed the best antioxidant performance in both the tested methods [39]. Reducing power assay of Petroleum ether extracts of *Eichhornia crassipes* (Mart.) Solms was studied at different concentrations and time delay and found to be increase with increase in concentration and time. The extracts were compared to standard antioxidant L-ascorbic acid. All the extracts showed greater reducing power than that of the standard [40].*In vitro* antioxidant testing of the extracts of *Samanea saman* (Jacq.) Merr in different solvents like petroleum ether, chloroform, ethyl acetate, aqueous and HCl was performed and found that mainly antioxidants were extracted in petroleum ether and had shown maximum reducing power [41] .

4. Conclusion:

Phytochemical screening of methanolic extracts of four herbs *Ocimum sanctum, Mentha spicata, Trigonella foenum-graecum, Spinacia oleracea* and one medicinally important tree *Gmelina arborea* had revealed the presence flavonoids, tannins, terpenoids, saponins, steroids, alkaloids by positive reaction with the respective test reagent. Results obtained in this investigation indicate that *Mentha spicata* leaf extract, rich in phenolics exhibited highest antioxidant and reducing activities. Total phenolic content had positive correlation with antioxidant capacity. It was observed that the leaf extract contained high level of phenolic content that might have accounted for the strong activity observed against DPPH radicals. The finding of this study suggests that this plant leaves could be a potential source of natural antioxidant that could have great importance as therapeutic agents in preventing or slowing the progress of ageing and age associated oxidative stress related degenerative diseases. Further investigation on the isolation and characterization of the antioxidant constituents is however required.

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