



Antioxidant Potential, Phenolics Content and Antimicrobial Attributes of Selected Medicinal Plants

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Abstract

The bioactive extracts recovered from *Curcuma longa* (stem), *Mentha aquatica* (stem and leaves), *Embolia officinalis* (fruits), *Nigella sativa* (seeds), and *Glycyrrhiza glabra* (stem) using methanol and ethanol, were appraised for antioxidant (total flavonoid contents, total phenolic contents, DPPH free radical scavenging ability, inhibition of linoleic acid peroxidation and reducing power) and antimicrobial attributes. Total phenolics (16.89 ± 0.18 - 25.06 ± 0.31 g GAE/100g) and total flavonoids (1.96 ± 0.07 - 13.54 ± 0.18 CE g/100g) contents of aqueous methanol extracts of tested plant materials were significantly ($p < 0.05$) lower than that of aqueous ethanol extracts (13.87 ± 0.12 - 28.63 ± 0.34 g GAE/100g) and (2.20 ± 0.10 - 8.71 ± 0.24 CE /100g), respectively. The percent inhibition of linoleic acid per oxidation by crude ethanol extracts of plants was in the range of 48.72 ± 1.24 - 70.79 ± 1.57 and crude methanol extract 35.90 ± 1.28 - 61.54 ± 2.14 , while the range of DPPH free radical scavenging activity of ethanol extracts was (58.36 ± 1.98 - 80.55 ± 3.07) and methanol extracts (39.55 ± 1.52 - 79.64 ± 2.33). The reducing power of the tested extracts obtained by ethanol (at the concentration of 10 mg/mL) ranged 1.11 ± 0.12 - 1.53 ± 0.18 while for methanol extracts 0.98 ± 0.11 - 1.39 ± 0.12 . Among the attributes analyzed, total phenolics, total flavonoids, and DPPH free scavenging activity were found to be varied significantly in all the tested medicinal plants. The extracts from all plants showed good antimicrobial potential against a panel of bacteria, including *E. coli*, *P. multocida* and *S. aureus*, and fungi including *A. niger*, *A. flavus*, *A. alternate*, and *G. lucidium*. Overall, the tested samples were found to be a good source of natural antioxidant and antimicrobial agents and thus can be explored for potential functional food and nutra-pharmaceutical applications.

Keywords: Medicinal flora, Antioxidant components, Solvent extraction, Colorimetric assays, Antimicrobial agents

Introduction

It is widely believed that antioxidants are the substances that have the ability to defend the body against oxidative damage that occurs due to free radicals [1]. In the food industry, antioxidants have been used to stop or slow

down the process of deterioration and enhance the shelf-life of foods, especially those containing polyunsaturated fatty acids [2]. Based on ethno-medicinal knowledge, many plants have been checked and employed for

the treatment of various diseases in folk medicines. Medicinal benefits of plants can be ascribed to the existence of a variety of phytochemicals so-called bioactive, with various biological and pharmacological activities [3-5]. Besides other bioactives, medicinal plants are also known as the richest source of natural phenolics, which exhibit free radical scavenging and antimicrobial potential [3,6].

Pakistan is blessed with a wide variety of medicinal flora, including herbs, shrubs, and trees with functional food/ nutraceutical potential and physiological benefits; several of such species are employed in the local/folk system of medicine for the treatment of different ailments [4-7].

Of the indigenous medicinal plant, typically, *Mentha aquatica* is an important organic herb from the family “*Lamiaceae*” and called “*water mint*”. Its extract, being a potential source of natural antioxidants, can reduce diphenyl picrylhydrazyl (DPPH) radicals in a concentration dependent manner [8]. Another important herb, *Curcuma longa* from the “*Zingiberaceae*” family, also called “*Turmeric*” has anti-carcinogenic potential due to the presence of antioxidants and free-radical scavenging agents [9]. *Emblica officinalis* belonging to the “*Phyllanthaceae*” family with the common name “*Gorse berry*” is also popular due to its medicinal and antioxidant effects [10]. Similarly, another local species *Glycyrrhiza glabra*, from a family of “*Fabaceae*” is valued for its antioxidant potential due to the presence of isoflavonoids and other flavonoid derivatives [11]. Black cumin (*Nigella sativa*), a member of “*Ranunculaceae*”, is reported to exhibit DPPH scavenging potential due to the presence of antioxidant compounds [12].

Although many indigenous plants from Pakistani flora possess biological and

medicinal potential, the plant materials selected in the present research work are conventionally used as folk medicine for the treatment of various diseases as well as employed in the formulations of different Ayurvedic medicines. So there is a prompt need to evaluate and provide a scientific basis towards elucidating and establishing the medicinal value of these species. Very limited reports are available on the antioxidant and antimicrobial properties of species selected for this study. In particular, the biological attributes of aqueous alcoholic extracts from different parts of selected species such as *Mentha aquatica*, *Glycyrrhiza glabra*, *Emblica officinalis*, *Curcuma longa*, and *Nigella sativa* native to Pakistan have not been assessed. Therefore, this research was framed to assess the antioxidant and antimicrobial attributes of aqueous methanol and aqueous ethanol extracts from different parts of the above medicinal plants.

Materials and Methods

Reagents and Chemicals

BHT (butylated hydroxytoluene) (99%), Linoleic acid, gallic acid, catechin, DPPH (Diphenyl picrylhydrazyl), and Folin-Ciocalteu reagent with the concentration of 2 N were bought from Sigma Chemicals Co. (USA). The additional chemicals used in this research were obtained from Merck (Germany) in pure form.

Samples

Three different samples of the stem of *Curcuma longa* (Haldi), stem and leaves for *Mentha aquatica* (Aabi Podina), stem of *Glycyrrhiza glabra* (Mulathi), fruits of *Emblica officinalis* (Amla), and seeds of *Nigella sativa* (Kalonji) were obtained from Department of Horticulture, University of Agriculture Faisalabad (UAF), Punjab, Pakistan, and the specimens were

authenticated by Department of Botany, University of Agriculture, Faisalabad. The samples were washed with deionized water and dried in a well-ventilated laboratory under ambient temperature conditions.

Preparation of Extracts

Dried samples of fruits of *Emblica officinalis*, stem and leaves of *Mentha aquatica*, stem of *Glycyrrhiza glabra*, stem of *Curcuma longa*, and seeds of *Nigella sativa* were pulverized to get fine powder with the particle size of 80 mesh using a commercial grinder (West point, France). Ten grams of the powdered samples were extracted with 100 mL of methanol: water (80:20 v/v) solution and ethanol:water (80:20 v/v) solution separately using an orbital shaker at room temperature for 8 h, and the insoluble residue was removed by filtration. The residue was again extracted two times using the same amount of fresh solvent, and all the three extracts for each category of solvent were combined. Evaporation of solvent was done using a rotary evaporator (EYELA, Japan) to concentrate the extracts under reduced pressure. These concentrated extracts were used to calculate the percentage yield and kept at -4°C for further experiments.

Antioxidant Activity of Medicinal Plants Extracts

Total phenolic contents

The TPC (total phenolic contents) of the plant extracts were investigated using Folin-Ciocalteu reagent procedure [13]. For this purpose, 50 mg of extract obtained from a plant was mixed with 0.5 mL of Folin-Ciocalteu reagent, followed by the addition of 7.5 mL of de-ionized water. Incubated the mixture for 10 min, then added 1.50 mL of 20% aqueous solution of sodium carbonate. Heated the above reaction mixture at 40°C in

a water bath for 20 min; finally the solution was cooled using an ice bath. The solution turned blue and noted the absorbance at the wavelength of 755 nm using a spectrophotometer (Hitachi, Japan, U-2001). The result of total phenolic contents was expressed in mg/gram of gallic acid equivalent of dry weight.

Total flavonoid contents

The TFC (total flavonoid contents) of plant extracts were measured using the method described by Dewanto et al. [14]. In this method, 0.1 mg of crude extract was combined with 4 mL water followed by addition of 0.30 mL 5% (w/v) solution of NaNO_2 along with 0.3 mL 10% (w/v) solution of AlCl_3 and 2 mL 1M solution of NaOH. Finally, 2.4 mL distilled H_2O was added into the reaction mixture and mixed completely. A spectrophotometer was used to record the absorbance at a wavelength of 510 nm, and contents of flavonoids were expressed as mg/grams of catechin equivalent of dry weight.

DPPH free radical scavenging assay

The scavenging ability of DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical measures the antioxidant capacity of plants extracts [15]. For this purpose, 0.5 – 100 $\mu\text{g/L}$ of crude extracts obtained from selected plants were mixed with 1.0 mL of 90 μM solution of DPPH then added 95% methanol to make the volume 4 mL. Butylated hydroxytoluene (BHT) was used as a positive control in this experiment. After the incubation time of 1 hour, the absorbance of the solution was measured at 515 nm. The percentage inhibition of DPPH free radical was measured by the formula given below:

$$I (\%) = 100 \times (A_b - A_s / A_b)$$

Here absorbance of control reaction mixture, which contain all the reagents except the sample extracts, was denoted by “Ab” and the absorbance of the tested extract was given by “As”.

Inhibition of linoleic acid peroxidation

The percent inhibition of linoleic acid peroxidation was assessed by the method previously reported by Iqbal *et al.* [16]. In this method, 5 mg of crude extract was mixed with 0.13 mL of linoleic acid followed by 10 mL of absolute ethanol (99.8%) and 10 mL of sodium phosphate buffer of concentration 0.2M and pH 7. Finally, distilled H₂O was mixed in the above reaction mixture to make the volume up to 25 mL and then incubated the solution for 360 hours at 40 °C. The oxidation strength was noted by measuring the peroxide value using thiocyanate method as reported earlier [17]. During this process, 75% ethanol solution in water (10 mL), 30% solution of ammonium thiocyanate in water (0.2 mL), 20 mM solution of ferrous chloride in 3.5% HCl (0.2 mL), and 0.2 mL sample solution was mixed to the pre-incubated reaction mixture. Stirred the solution for 3 minutes, and absorbance was noted at the wavelength of 500 nm using a spectrophotometer. A control reaction mixture was also checked under the same experimental condition but without extracts. BHT, a synthetic antioxidant, was used as a positive control. The inhibition capacity of linoleic acid peroxidation in terms of percentage was measured using the formula:

$$\% = 100 - \left[\frac{\text{increase in absorbance (sample)}}{\text{increase in absorbance (control)}} \times 100 \right]$$

Reducing power

This antioxidant assay of plant derived extracts was accessed by using the method written by Sultana *et al.* [18]. Different amount

of extract obtained from plants ranging from 2.5 to 10.0 mg was combined with 5.0 mL of phosphate buffer with concentration 0.2 M having pH 6.6 and 5.0 mL of 1.0% solution of potassium ferricyanide in water. Incubated the above reaction mixture for about 20 minutes at 50 °C, then added 5 mL of 10% solution trichloroacetic acid. Centrifuged the reaction mixture at 5 °C for 10 minutes at the speed of 80 rpm in a low temperature centrifuge machine (Kokusan Denki, Japan, CHM-17). Took 5.0 mL of the upper layer, diluted it with 5.0 mL of distilled H₂O, then mixed with 1.0 mL of freshly prepared 0.1% solution of ferric chloride in water. Noted the absorbance of the reaction mixture at 700 nm.

Antimicrobial Activity

All the extracts of selected medicinal plants were tested separately for antimicrobial activity against three bacterium strains *E. coli*, *P. multocida* and *S. aureus* and four fungal stains *A. niger*, *A. flavus*, *A. alternata*, and *G. lucidium*. All these micro-organism including bacteria and fungus, were procured from Biochemistry Department of University of Agriculture, Faisalabad, Pakistan. For the optimum growth of selected micro-organisms, the bacteria used in the present research work were cultured at an optimum temperature of 37 °C on nutrient agar (Oxoid, UK), and the fungus was cultured at an optimum temperature of 30 °C on potato dextrose agar (Oxoid, UK).

Disc diffusion method

The selected plant extracts were subjected to analyze the antimicrobial activity using the disc diffusion method [19]. For this purpose, small discs with 6 mm of diameter were dipped with sample extract (50 µL) and placed on tested micro-organism previously inoculated on the agar plate. For comparative results, Rifampicin and Fluconazol with the

amount of 30 µg/disc were taken as positive reference standard drugs for bacterial and fungal strain, respectively. The whole setup was incubated for 24 h at 37°C for bacterial growth and 3 days at 25 °C for fungal growth. The antimicrobial activity of tested extracts was accessed by measuring the zone of inhibition of microorganism growth in millimeters.

Micro dilution broth assay

The MIC (minimum inhibitory concentration) value of plant extracts was checked using the microdilution broth method previously reported [19]. On a 96-well microtiter plate, a sequence of dilution of plant extracts was placed, followed by two controls, i.e., growth control and sterility control. For bacterial strain 160 µL of NB and for fungal strain SBD were combined with 20 µL of tested solution into the microplates. Then inoculated the plates with the suspension of standard micro-organism (20 µL of 5×10^5 CFU/mL). Incubated the plates for 24 h at 37°C for the bacterial strain and 48 h at 28°C for fungal strain. The reference compound Rifampicin was used as an antibacterial and Fluconazole as an antifungal agent. At the bottom of the well, white pellets were produced, which indicated the growth, and the results were reported in the form of MIC value (mg/mL).

Statistical Analysis

Each experiment was performed three times to compute three measurements (replicates). The experimental data were analyzed statistically using a statistical tool/software STATISTICA 5.5 (USA). The significant difference between different samples was given by a probability value of $p \leq 0.05$. The whole data was recorded as mean values \pm SD (Standard deviation) for triplicate determinations [20].

Results and Discussion

Percentage (%) Yield of Medicinal Plants Extracts

The yield of extractable antioxidant components (g/100 g of DW) from different parts of selected plants with 80% aqueous solution of methanol and 80% aqueous solution of ethanol as shown in Fig.1 varied as a function of extraction solvents and plants part used. It can be noted that maximum extract yield (33.29 g/100g of DW) was obtained from *E. officinalis* with aqueous ethanol (80%) while aqueous methanol (80%) gave the lowest yield (12.72 g/100g of DW) from *C. longa*. The order of maximum percentage yield with aqueous ethanol (80%) was found to be *E. officinalis* > *G. glabra* > *N. sativa* > *M. aquatica* > *C. longa*. A significant ($p < 0.05$) variation is observed in the present research work for the percentage yield of extracts among the tested medicinal plants. As far as the efficacy of the extraction solvents is concerned, the yield of extracts (g/100g of DW) from different parts of the selected plants ranged from 12.72 to 23.38% and 14.50 to 33.29% for aqueous methanol and aqueous ethanol, respectively.

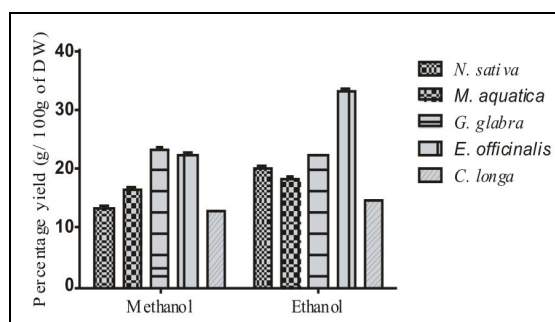


Figure 1. Percentage extract yield (g/100g of DW) from selected medicinal plants with different solvent systems. Values (mean) are average of three samples of each medicinal plant material, analyzed individually in triplicate ($n = 1 \times 3 \times 3$), ($P < 0.05$).

Alcohols like methanol and ethanol are used as solvents for the recovery of potent antioxidant compounds from different plants tissues due to their solubility [18,21,22]. In

line with the earlier reports [18,21,22], the present findings showed the higher yield of extract from different plant materials with 80% aqueous solution of ethanol, which resulted in the higher efficiency of this solvent to recover bioactive compounds. Furthermore, our findings also showed a significant ($p < 0.05$) difference in the amount of extracts from selected medicinal plants, which can be linked to the recovery of various extractable antioxidant compounds into the extraction solvents.

The chemical nature of the various bioactives may also affect the percentage yield of extracts with different solvents [23]. For extracting biological components from different parts of the plants, aqueous solutions of methanol and ethanol were found to be effective solvents, but out of these two, aqueous solution of ethanol is preferred due to its non-toxic and green solvent nature. [24].

The higher percentage yield using aqueous ethanol solution in this research work is in agreement with the findings of Ahmad *et al.*, [25], who recorded the higher percentage yield of extracts from flowers and leaves of Akk (*Calotropis procera*) using ethanol as solvent. The total amount of bioactives/antioxidants obtained from different plant materials depends on multiple factors like availability of components, chemical nature, and technique used to get extracts [23].

Total Phenolic Contents

Due to the potential health benefits, phenolic contents are becoming an important ingredient for functional foods and nutraceutical industries [26]. Plants derived phenolic contents can be used as natural antioxidants [27]. A large number of reports suggest that the phenolic compounds present

in fruits, vegetables, and food plants contribute mainly toward their antioxidant activity [28]. In the present case, measurement of TPC in different parts of selected medicinal plant extracts was performed using Folin-Ciocalteu method, and the result was recorded and GAE (Gallic Acid Equivalent) [13]. TPC of different medicinal plants ranged from 16.89 to 25.06 and 13.87 to 28.63 g GAE/100g for extracts in aqueous methanol and aqueous ethanol, respectively (Fig. 2). A significant difference ($p < 0.05$) for TPC was noted within different plants. Aqueous ethanol extracts from some of the selected medicinal plants offered a comparatively higher amount of TPC in comparison to aqueous methanol extracts. The highest amount of TPC was shown by aqueous ethanol extract of *M. aquatica* followed by *G. glabra* > *N. sativa* > *E. officinalis* > *C. longa*. Total phenolics (18.47-19.04) in *N. sativa* in this research work are quite similar to the findings of Bourgou [29].

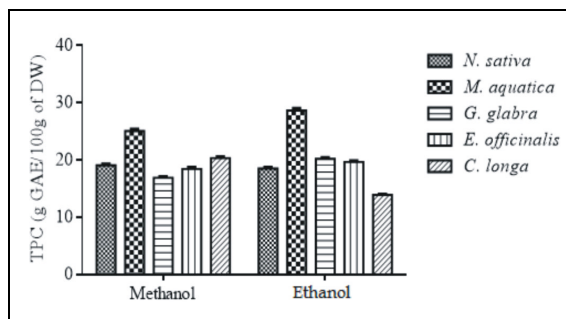


Figure 2. Total phenolic contents (g GAE/100g of DW) of extracts from selected medicinal plants with different solvent systems. Values (mean) are average of three samples of each medicinal plant material, analyzed individually in triplicate ($n = 1 \times 3 \times 3$), ($P < 0.05$)

TPC present in the extracts of different medicinal plants varied significantly ($p < 0.05$) with reference to the extracting solvent used and the part of the plant used. For the recovery of food grade biologically active compounds from plant materials, ethanol is relatively preferable due to its non-toxic nature, green nature, and good extraction efficiency [24,30].

Different plants showed a different amount of phenolic contents due to the fact that the presence of bioactive compounds in plants varied by plants, soil condition, maturity, and treatment after harvesting [31, 47]. For example, TPCs lower than our present values were reported in *corn cob*, *Moringa oleifera* leaves, *Eugenia jambolana* bark, *Ficus religiosa* fruit, and *Aloe barbadensis* leaves [18,32]. Likewise, TPCs higher than our present investigations were reported in *Gmelina* leaf, *Kigelia* stem, *Hibiscus* fruit [6]. Moreover, some studies showed that the composition and amount of phenolics varied within the tissues at a sub-cellular level [33].

Total Flavonoid Contents

Total flavonoids contents (TFC) determined as Catechin Equivalent (CE) in the extracts of different parts of selected medicinal species ranged from 1.96 to 13.54 and 2.20 to 8.71 CE /100g respectively (Fig 3). The amount of TF in tested plant extracts varied significantly ($p < 0.05$) within the species studied. Total flavonoids in aqueous ethanol extracts of different plant species were found to be higher than aqueous methanol extracts. The highest amount of TFC was noted in aqueous ethanol extract of *M. aquatica* followed by *C. longa* > *E. officinalis* > *N. sativa* > *G. glabra*.

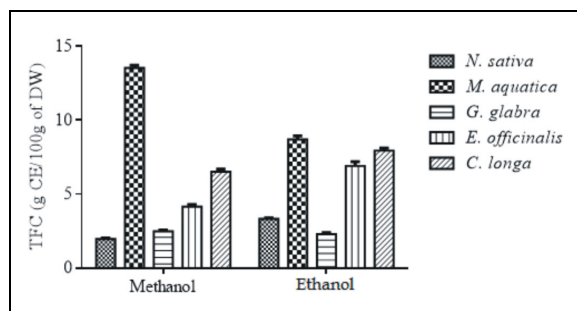


Figure 3. Total flavonoid contents (g CE / 100 g of DW) of extracts from selected medicinal plants with different solvent systems. Values (mean) are average of three samples of each medicinal plant material, analyzed individually in triplicate ($n = 1 \times 3 \times 3$), ($P < 0.05$).

The amount of total flavonoids in different parts of selected medicinal plants was found to be higher than the findings of Ebrahimzadeh *et al.*, [34], who estimated TF in *V. odorata* leaves, *B. hyrcana*. In contrast, it was lower than the findings of Doss *et al.*, [35], who determined TFC in *Canavalia gladiata* and *Canavalia ensiformis*. The ratio between total flavonoids and total phenolics varied significantly in different plant species as reported by Ribarova and Atanassova [46]; they studied the ratio between total flavonoids and total phenolics in different Bulgarian fruits and vegetables.

DPPH Radical Scavenging Activity

The free radical capturing/scavenging assay is designed on a mechanistic approach wherein the radical neutralization ability of extract is estimated colorimetrically. DPPH free radical, used in this research work, is an organic free radical that is stable, has a deep violet color, and exhibits maximum absorption within the wavelength range of 515-528 nm [36]. During this reaction, the deep violet color of DPPH is converted into yellow color as its chromophore is deactivated upon receiving a proton from plant phenolics, act as a hydrogen donor. It is a well-known fact that the DPPH free radical capturing activity varies directly with the amount of phenolics (hydrogen donors) or the extent of hydroxylation of phenolic compounds, hence antioxidant potential of plant derived extracts increases by the higher concentration of phenolics [37]. Lipid oxidation is a serious issue of the food industry and leads to loss of nutritional value of products and economic losses, and as a result of oxidative stress, free radicals are produced in biochemical processes in the body, so the antioxidant molecules slow down/interrupt oxidation process by scavenging/neutralizing free radicals or chelating the transition metals [38]. Inhibition of the chain initiation step in the process of oxidation is done by scavenging the

reactive oxygen species and free radicals, this mode of action is considered an important antioxidant method [39].

DPPH free radical capturing ability of selected medicinal plant extracts by aqueous methanol and aqueous ethanol showed a noticeable scavenging activity in the range of 39.55 to 80.64 and 58.36 to 80.55, respectively (Fig. 4). The aqueous ethanol extract of *E. officinalis* showed maximum DPPH free radical capturing activity, while methanol extracts of *C. longa* scavenged the minimum DPPH free radical.

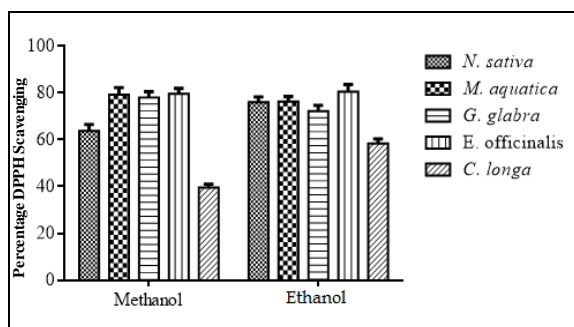


Figure 4. DPPH radical scavenging activity of extracts from selected medicinal plants with different solvent systems. Values (mean) are average of three samples of each medicinal plant material, analyzed individually in triplicate ($n = 1 \times 3 \times 3$), ($P < 0.05$)

Free radical capturing ability changed significantly ($p < 0.05$) among the selected medicinal plants. The antioxidant activity is increased due to the enhanced free radical capturing/scavenging ability with a greater amount of phenolic contents [37]. Plant phenolic compounds, being hydrogen donors, can be linked to quenching DPPH radicals. In a previous study, similar findings to our present results have been exhibited by the extract of leaves of *Lantana camara* (L) [40].

Inhibition of Linoleic Acid Peroxidation

The polyunsaturated fatty acids (PUFA), like linoleic acid (C18:2), produce peroxides upon oxidation. These peroxides have the ability to oxidize Fe^{2+} to Fe^{3+} ; and

then form a complex with SCN. The intensity of the color formed by the complex can be related to the concentration, which can be noted by measuring the absorbance at the wavelength of 500 nm. The greater the concentration of peroxides generated in the reaction mixture greater is the value of absorbance and hence results in the lower antioxidant activity [17].

The measure of prevention of lipid oxidation can be used to access the antioxidant ability of plant derived extracts. The assay was done by finding out the ability to inhibit the peroxidation of linoleic acid by thiocyanate method [17]. For the comparison of our result of antioxidant activity by the extracts of selected five medicinal plants BHT was used as a positive control.

The strength of peroxidation inhibition (%) of different plant extracts in linoleic acid for 15 days (360 h) incubation period as depicted in Fig. 5 showed different levels of inhibition, ranging from 35.90 to 61.54 % and 51.2 to 71.79% for an aqueous solution of methanol and aqueous solution of ethanol extracts, respectively. The inhibition of peroxidation, hence antioxidant activity, varied significantly ($p < 0.05$) among different plant extracts tested. Maximum activity was observed for extracts obtained by aqueous ethanol solution of *G. glabra* followed by *M. aquatica* > *N. sativa* > *E. officinalis* > *C. longa*.

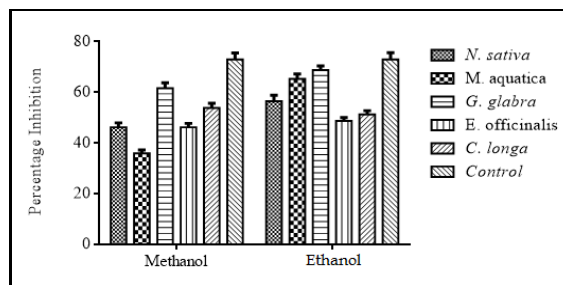


Figure 5. Percent inhibition of linoleic acid peroxidation of extracts from selected medicinal plants with different solvent systems. Values (mean \pm SD) are average of three samples of each medicinal plant material, analyzed individually in triplicate ($n = 1 \times 3 \times 3$), ($P < 0.05$)

The results of antioxidant activity of *G. glabra* in the present case are quite comparable with those reported for lipid peroxidation of ethanolic extracts of the same species by Visavadiya *et al.*, [41]. Meanwhile, the present percent inhibition values are higher than the values obtained for some other potential medicinal plants such as *Biebresteinia multifida*, *Astragalus hamosus*, *Salvia hydrangea*, and *Zataria multiflora* [3,42].

Reducing Power

This assay is based on the ability of electron transfer or hydrogen atom transfer via redox potential of the antioxidant extracts/compounds [39]. The reducing ability of phytochemicals present in the extracts of plants varies directly with the antioxidant activity. Thus, accessing the reducing power of extracts can be another method to measure the antioxidant activity.

The reducing power of extracts from different medicinal plants (shown in Fig. 6) was measured over the extract concentration range of 2.5 – 10.0 mg/mL. A general trend of direct relationship between reducing power and concentration of extracts was observed. Reducing potential of extracts of different plants by both solvents ranged from 0.54 to 1.23 and 0.68 to 1.52, respectively.

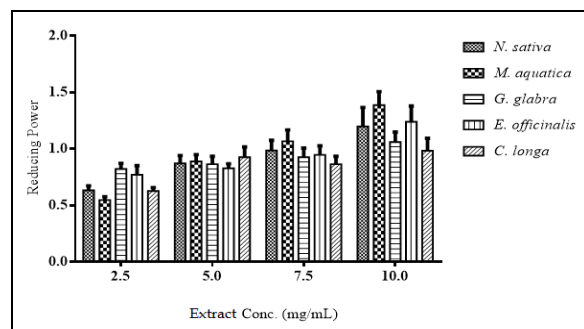


Figure 6. Reducing power of methanolic extracts from selected medicinal plants. Values (mean) are average of three samples of each medicinal plant material, analyzed individually in triplicate ($n = 1 \times 3 \times 3$), ($P < 0.05$).

The nature of the solvent used for the extraction also have a significant ($p < 0.05$) effect on the reducing ability of the extracts. The data obtained in the present study for reducing power of extracts followed the same trends as reported in previous studies [18,25].

Antimicrobial Activity

Disc diffusion method

The antimicrobial activity of selected medicinal plants derived extracts were measured using disc diffusion method against a panel of bacteria (*E. coli*, *S. aureus*, and *P. multocida* strains) and fungi (*A. flavus*, *A. niger*, *A. alternata* and *G. lucidum*) and inhibition zones were measured in millimeter (mm) (Table 1). It is clearly shown in data that ethanol extract of *Nigella sativa* showed strong activity (25 mm zone of inhibition) against *E. coli* while aqueous methanol extract of *Mentha aquatica* showed minimum bactericidal activity (13 mm zone of inhibition). Aqueous ethanol extract of *Curcuma longa* showed strong activity (29 mm zone of inhibition) against *A. alternata*, while aqueous ethanol extracts of *Mentha aquatica* gave the poorest fungicidal effect against *A. niger* (12 mm zone of inhibition). Generally, all the plant species used in the present research work showed minimum to maximum antimicrobial activity against the panel of microorganism used.

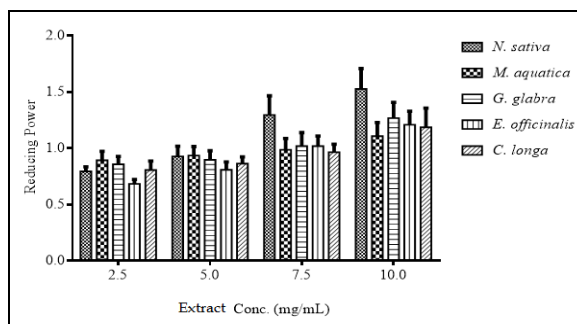


Figure 7. Reducing power of ethanolic extracts from selected medicinal plants. Values (mean) are average of three samples of each medicinal plant material, analyzed individually in triplicate ($n = 1 \times 3 \times 3$), ($P < 0.05$).

Table 1. Antimicrobial activity of methanol and ethanol extracts from selected medicinal plants.

Microorganisms		<i>Mentha aquatica</i>	<i>Curcuma longa</i>	<i>Embllica officinalis</i>	<i>Glycyrrhiza glabra</i>	<i>Nigella sativa</i>
Bacteria		Zone of inhibition (mm)				
<i>E. coli</i>	Methanol Extract	13.00 ±0.04	20.00 ±0.09	20.00 ±0.02	21.00 ±0.01	14.00 ±0.01
<i>P. multocida</i>		20.00 ±0.04	17.00 ±0.01	19.00 ±0.03	17.00 ±0.04	16.00 ±0.01
<i>S. aureus</i>		19.00 ±0.01	14.00 ±0.02	22.00 ±0.04	20.00 ±0.02	18.00 ±0.05
<i>E. coli</i>	Ethanol Extract	21.00 ±0.02	17.00 ±0.01	20.00 ±0.01	23.00 ±0.02	25.00 ±0.03
<i>P. multocida</i>		22.00 ±0.04	18.00 ±0.01	19.00 ±0.04	22.00 ±0.03	19.00 ±0.03
<i>S. aureus</i>		17.00 ±0.02	23.00 ±0.01	20.00 ±0.03	19.00 ±0.04	21.00 ±0.02
Fungi		Zone of inhibition (mm)				
<i>G. lucidum</i>	Methanol Extract	22.00 ±0.02	17.00 ±0.04	18.00 ±0.02	23.00 ±0.04	16.00 ±0.03
<i>A. flavus</i>		16.00 ±0.03	17.00 ±0.02	13.00 ±0.04	14.00 ±0.01	15.00 ±0.02
<i>A. niger</i>		12.00 ±0.01	16.00 ±0.04	15.00 ±0.03	16.00 ±0.04	14.00 ±0.02
<i>A. alternate</i>		18.00 ±0.04	22.00 ±0.02	24.00 ±0.01	27.00 ±0.02	19.00 ±0.01
<i>G. lucidum</i>	Ethanol Extract	20.00 ±0.03	24.00 ±0.03	22.00 ±0.01	19.00 ±0.04	18.00 ±0.03
<i>A. flavus</i>		16.00 ±0.02	15.00 ±0.01	19.00 ±0.04	17.00 ±0.02	14.00 ±0.01
<i>A. niger</i>		17.00 ±0.03	19.00 ±0.03	18.00 ±0.01	20.00 ±0.01	16.00 ±0.04
<i>A. alternate</i>		21.00 ±0.01	29.00 ±0.02	20.00 ±0.01	23.00 ±0.04	25.00 ±0.01

Note: Data are the mean of three independent replicates, rounded off to near whole numbers. Values are mean \pm SD of three samples analyzed individually in triplicate at $p < 0.05$.

The difference in the antimicrobial activity of selected parts of medicinal plant extracts may be due to the difference in the composition of extracts by different plants. Previous studies also reported that the biological activity of the extracts obtained from different plants is directly affected by the change in their chemical composition [43,45]. The antimicrobial activities of the plant species are due to the presence of certain bioactive phytochemicals such as ascorbic acid, saponins, flavonoids, betulinic acid, phenolic acids and glycosides. Alkaloids are the class of strong antibacterial agents, and *Curcuma longa* contains alkaloids such as coclaurine, franguloline, norisoboldine, isoboldine, iusirine, asimilobine, and iusiphine. The Antimicrobial potential of roots of *Glycyrrhiza glabra* was reported less than our present findings by [44].

Minimum inhibitory concentration (MIC)

This is the concentration of extracts that can completely limit the growth capability of microorganisms. For measuring the minimum inhibitory concentration (mg/mL) of extracts, the microdilution broth susceptibility assay was used [19]. Table 2 revealed that aqueous ethanol extract of *Curcuma longa* showed the lowest minimum inhibitory concentration (12 mg/mL), indicating its best activity against *S. aureus* while aqueous methanol extract of *Mentha aquatica* had the highest minimum inhibition concentration (67 mg/mL), showing the weakest activity against *E. coli*. Aqueous ethanol extract of *Curcuma longa* exhibited the lowest minimum inhibition concentration (11 mg/mL), showing greater effectiveness against *A. alternate*, while aqueous methanol extract of *Mentha aquatica* had a higher minimum inhibition concentration (83 mg/mL), indicating its weakest against *A. niger*.

Conclusion

Based on the results of different assays, it was noted that aqueous ethanol extracts from selected parts of different medicinal plants exhibited relatively better antioxidant activity linked to appreciable amounts of TP and TF in these extracts. All the tested extracts showed appreciable but variable antimicrobial activity. In general, extraction of antioxidant / antimicrobial agents by different solvents from selected parts of medicinal plants yielded extracts of different chemical nature and hence showed different biological activities. This difference can be due to the availability of various components depending upon the solvent used for extraction and the part of the plant used. Therefore, it can be concluded that a suitable extraction process must be selected to recover a good yield of powerful antioxidant compounds from the selected parts of the subject medicinal plants. Additional detailed analysis is recommended to characterize individual bioactives and study the structure-activity relationship of these compounds and in vivo assessment of biological activities trials of the major component in different parts of the selected medicinal plants.

Conflict of Interest

The authors state that they have no conflict of interest.

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