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Original Article

Evaluation of Antioxidant Activity of Four Locally Grown Aromatic Herbs Commonly Consumed in Bangladesh

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ABSTRACT: Considerable interest has been given on dietary antioxidants with reference to their protective role against oxidative damage. The current study is designed to evaluate the antioxidant activity of four aromatic herbs generally consumed in Bangladesh *i.e. Mentha viridis*, *Mentha arvensis*, *Coriandrum sativum* and *Eryngium foetidum*. Therefore, total phenolic content, total flavonoid content and radical scavenging activity are determined by DPPH assay. The highest phenolic and flavonoid content is observed in *Mentha viridis* which is 100.67±2.08 mg GAE/g sample extract and 384.21±1.81 mg RUE/g sample extract respectively and the lowest in *Eryngium foetidum*. The antioxidant activity (percent inhibition) is the highest in *Mentha arvensis* followed by *Mentha viridis* with increasing concentrations ranging between 3µg/mL-100µg/mL and showed better activity than standard ascorbic acid. *Mentha arvensis* exerted the minimum IC_{50} value (26.21±2.08 µg/mL) which is in accordance with its maximum DPPH radical scavenging activity. Total phenolic and flavonoid content are positively related with the radical scavenging percentage of *Coriandrum sativum* and *Eryngium foetidum* whereas *Mentha viridis* and *Mentha arvensis* are not significantly related. It might be concluded that polyphenols, flavonoids are main components but other phytochemicals could contribute towards radical scavenging activity. However, studied materials altogether posses good antioxidant activity.

KEYWORDS: Antioxidant, Polyphenols, Flavonoids, Ascorbic acid, DPPH

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INTRODUCTION

Oxygen-centered free radicals and other ROS (reactive oxygen species), that are continuously produced *in vivo* as by products, cause cell death and tissue damage that may be linked with aging and diseases, for example atherosclerosis, diabetes, cancer and cirrhosis (1) . Though humans and other organisms feature antioxidant defenses (enzymes, such as superoxide dismutase and catalase, or compound such as ascorbic acid, tocopherols and glutathione) and repair systems that have evolved to guard them against oxidative damages, these systems are inadequate to totally defend the damage (2a,2b). Consequently, intake of antioxidant rich food is the most efficient way of combating different disorders and health risks. In recent years, a multitude of studies have been carried out on the antioxidant activity of different foods of plant origin and their derived products. Antioxidants obtained from plants are of greater benefit in contrast to synthetic ones since synthetic antioxidants (*e.g.* butylhydroxianisole, butylhydroxytoluene, gallates *etc*.) were found to exert genotoxic effects (3a and 3b). The protective

effects of plant products are due to the presence of several components which have seperate mechanisms of action; some are macromolecules e.g. enzymes and proteins and others are micro molecules such as vitamins, flavonoids and other phenolic compounds (4a, 4b, 4c, 4d and 4e). During antioxidant activity study, a focus has been placed on determination of plant derived polyphenols because; phenolic compounds act as radical scavengers, reducing agents and chelators of metal ions (5). Flavonoids a group of polyphenolic compounds with known properties, which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action (6) might be considered as a part of antioxidant activity determination. To measure total antioxidant capacity a rapid, simple and inexpensive method have been developed which involves the free radical scavenging assay of 2,2- Diphenyl-1- Picrylhydrazyl (DPPH) stable radical. In the presence of an antioxidant, DPPH radical obtains one free electron and the absorbance decreases (7). In a developing country like Bangladesh which has abundant plant resources providing the mass people with available food products having better health benefit is always appreciated. A number of culinary herbs are consumed as condiments as well as flavoring vegetarian and non vegetarian curries in Bangladesh. Among those most common are mint (local name- pudina) and coriander (local name- dhonia). Perennial aromatic herb mint (*Mentha* spp. Family- Lamiaceae) is cultivated in Bangladesh mainly for culinary purposes which is widely used by food and pharmaceutical industries as a flavor all over the world (8). The herb is considered as stimulant, carminatives, antispasmodic, stomachic and diuretic, and it is also used for gas pain, rheumatism, toothache, muscle pain and mouth wash (9). The genus consists of more than twenty five species and in Bangladesh two species: *Mentha viridis* synonym *Mentha spicata* Linn (spearmint) and *Mentha arvensis* L. (Japanese mint) are generally grown (10). *Mentha* extract has been found to have antioxidant and antiperoxidant properties due to the presence of eugenol, caffeic acid, rosmarinic acid and α-tocopherol (11) and it could enhance error-free repair for DNA damage and hence could be antimutagenic (12). Two different species namely *Coriandrum sativum* L. (common name coriander or cilantro; local name "dhania") and *Eryngium foetidum* L. (common name culantro; local name "belati dhania") belongs to the same family Apiaceae are available in our local markets. *Coriandrum sativum* L. is an annual herbaceous plant originally from the Mediterranean and Middle Eastern regions, cultivated for its culinary, aromatic and medicinal use (8). The essential oil and various extracts from coriander have been shown to possess antibacterial, antidiabetic, anticancerous, antimutagenic and antioxidant activities (11 and 12). Coriander fruit is also reputed as refrigerant, tonic, diuretic and aphrodisiac, while, the oil is considered useful in flatulent colic, rheumatism, neuralgia, etc. *Eryngium foetidum* L. is a tropical perennial and annual herb native to Mexico and South America, but is cultivated worldwide. Most of the times fresh leaves are used as a substitute for cilantro, but it have a much stronger taste. Its leaves and roots decoction are used for flu, pneumonia, diabetes, and constipation, where herb also has been used traditionally for treatment of fevers, vomiting, diarrhoea. Also analgesic, antimalarial and antibacterial properties were reported from traditional use (13a and 13b). Though a number of researchers have reported the antioxidant activity of *Mentha viridis* and *Coriandrum sativum* no reports have been available on *Mentha arvensis* and *Eryngium foetidum* from Bangladesh. Therefore, the current research is aimed to evaluate and compare the antioxidant potential of these four common aromatic and culinary herbs available in Bangladesh.

MATERIALS AND METHODS

Plant Materials

The four plant materials namely *Mentha viridis*, *M. arvensis*, *Coriandrum sativum* and *Eryngium foetidum* were purchased from local market of Dhaka city. All the materials were authenticated by the

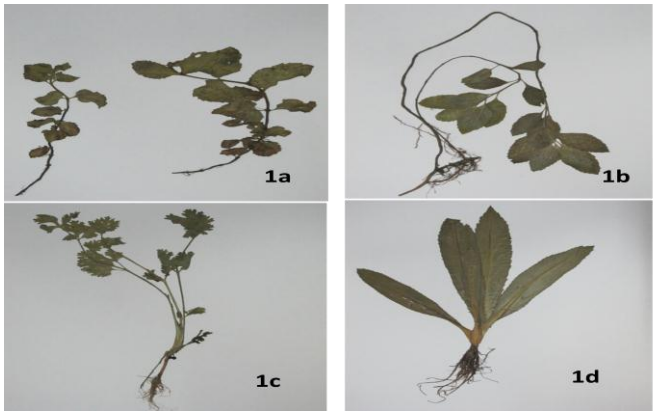

Figure 1. Herbarium specimen of studied materials namely *Mentha viridis* (a), *Mentha arvensis* (b), *Coriandrum sativun* (c) and *Eryngium foetidum* (d).

taxonomists of Dhaka University Salar Khan Herbarium, Department of Botany, University of

Dhaka. Edible portion i.e. leaves were used for experimental study (Fresh weight 250g for each material) and cleaned properly with tap water. Then dried in room temperature (around 30º C) under shed for three days and oven dried at 80º C for thirty minutes before powdered in a grinder machine. The botanical names, English names, family names, parts used and voucher specimen numbers are presented in Table 1. Powdered plant materials were kept in air tight container in dark and cool place.

Preparation of sample extracts

About 10g of powdered sample from each plant material was extracted with 250mL of 99% methanol in an orbital shaker for forty eight hours. Methanol has been reported to be the most suitable solvent in the extraction of polyphenolic compounds from plant tissue due to its ability to inhibit the action of polyphenol oxidase that causes the oxidation of polyphenols (14). The extracts were filtered using Rotilabo®- round filters, type 601A (125 mm) and concentrated on a rotary evaporator at around 40º C. Percent recovery of the four plant materials were as follows: *Mentha viridis*-8% *Mentha arvensis*-6% *Coriandrum sativum*-15% and *Eryngium foetidum*-13%. Concentrated extracts were stored in 4º C for further analysis.

Table 1. Features of the used plant materials

Botanical name	English name	Family name	Parts used	Voucher specimen no.
Mentha viridis	Spearmint	Lamiaceae	Leaves	DUSH 1788
Mentha arvensis	Japanese mint	Lamiaceae	Leaves	DUSH 1789
Coriandrum sativum	Coriander/Cilantro	Apiaceae	Leaves and shoots	DUSH 1790
Eryngium foetidum	Culantro	Apiaceae	Leaves	DUSH 1791

Estimation of total phenolic content

The content of total phenol in methanolic extracts of different samples was determined spectrophotometrically using Folin-Ciocalteu reagent (15) with modifications. Calibration curve was prepared by mixing methanolic solution of gallic acid (1 mL; 50, 100, 150, 200, 250 µg/mL) with 5mL Folin-Ciocalteu reagent (diluted tenfold with distilled water) and sodium carbonate solution in distilled water (4mL, 0.7 M). The absorption was measured at 765 nm using a Shimadzu UV-1800 spectrophotometer after incubating in 40° C for thirty minutes. One mL of plant extracts was mixed instead of 1mL gallic acid with the same reagents as described above to determination of the total phenolic contents. The absorbance was measured against a reagent blank, which was composed of the same reagents except test extract. Total content of phenolic in the plant extracts were expressed as gallic acid equivalents (mg of GAE/g sample) and were calculated by the formula:

$T = (C \times V) / M$

Where, T=total content of phenolic compounds (mg of GAE/g sample)

C=the concentration of gallic acid established from the calibration curve (mg/mL)

V= volume of extract (mL)

M= weight of methanolic plant extract (g)

Estimation of total flavonoid content

Total flavonoid content was estimated by Aluminum Chloride Colorimetric Method (16). To 1 ml of plant extract or standard of different concentrations 3 mL methanol, 0.2 mL of 10% aluminum chloride, 0.2 mL potassium acetate (1M) and 5.6 mL of distilled water were added. Then the solution was incubated for 30 minutes at room temperature. The absorbance was measured at 415 nm using Shimadzu UV-1800 spectrophotometer against a blank. Standard curve was prepared using rutin hydrate by dissolving it in methanol followed by serial dilution to 25, 50, 100, 200,400 µg/mL. Total content of flavonoid in the plant extracts were expressed as rutin hydrate equivalents (mg of RUE/g sample) and were calculated by the formula:

$T = (C \times V) / M$

Where, T = total content of flavonoid compounds (mg of RUE/g sample)

C = concentration of rutin hydrate established from the calibration curve (mg/mL)

V = volume of extract (mL)

M = weight of methanolic plant extract (g)

Estimation of DPPH free radical Scavenging Activity

The DPPH assay was performed as described by Blois (17) with minor modifications. In brief, 0.1 mM solution of DPPH in methanol was prepared. This solution (1 mL) was added to 2 mL of different extracts in methanol at different concentration (3, 6, 12.5, 25, 50, 100 µg/mL) which were prepared by dilution method. The solution was shaken well and incubated in the dark for 30 min at room temperature. The absorbance was subsequently recorded at 517 nm using Shimadzu UV-1800 spectrophotometer. The

scavenging activity was estimated based on the percentage of DPPH radical scavenged using the following equation:

DPPH scavenging effect (%) or Percent inhibition = A0 - A 1 / A0 × 100.

Where A0 was the Absorbance of control reaction and A1 was the Absorbance in presence of test or standard sample [18]. 1 mL of methanol in place of the sample extract along with DPPH was measured as the control. Reference standard compound being used was ascorbic acid and all tests were performed in triplicate.

RESULT AND DISCUSSION

Phenolic compounds are extensively distributed in plants that have gained great attention, due to their antioxidant activities and free radical-scavenging abilities, which potentially have beneficial

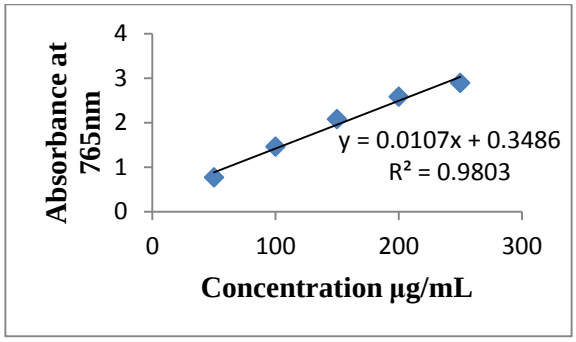

Figure 2. Standard calibration curve of gallic acid at concentrations of 50,100,150, 200 and 250µg/mL. Spectrophotometric detection was at 765nm.

implications for human health (19). The total phenolic content (TPC) was determined in

Table 2. Total phenolic content, total flavonoid content and IC_{50} values of plant methanolic extracts Results are the average of triplicate measurements±standard deviation.

Plant materials	Total phenolic content (mg GAE/g of sample)	Total flavonoid content (mg RUE/g of sample)	IC50 (µg/mL)
Mentha viridis	100.67±2.08	384.21±1.81	39.38±3.05
Mentha arvensis	66.00±2.00	295.68±2.85	26.21±2.08
Coriandrum sativum	15.33±2.08	100.91±0.90	68.14±5.10
Eryngium foetidum	6.66±0.53	62.00±1.32	120.315±4.50

IC_{50} values obtained from linear regression analysis of concentration vs. DPPH scavenging activity% . IC_{50} value for ascorbic acid was 48.05±2.1 µg/mL.

comparison with standard gallic acid and the results were expressed in terms of mg gallic acid equivalent (GAE)/ g of sample extract. The total phenol content of the methanolic extract of studied four herbs in terms of gallic acid equivalent were between 100.67 mg GAE/g of sample and 6.66 mg GAE/g of sample (the standard curve equation y=0.010x+0.348, r^2=0.980 Figure 2). The total phenolic content was observed in four materials

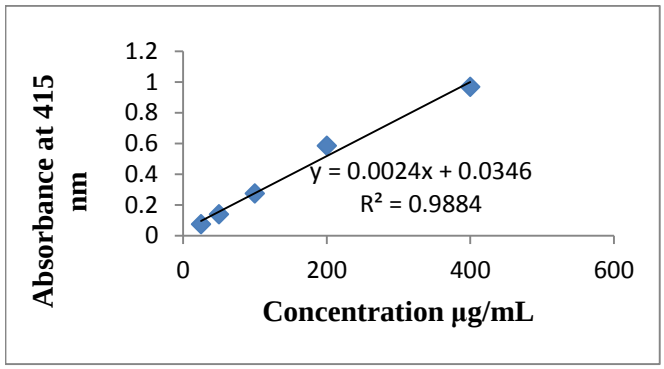

Figure 3. Standard calibration curve of rutin hydrate at concentrations of 25,50,100,200 and 400 µg/mL. Spectrophotometric detection was at 415nm.

extract as: *Mentha viridis*>*Mentha arvensis*>*Coriander sativum*>*Eryngium foetidum* (Table 2). It is known that flavonoids with a certain structure and particularly hydroxyl position in the molecule can act as proton donators and show

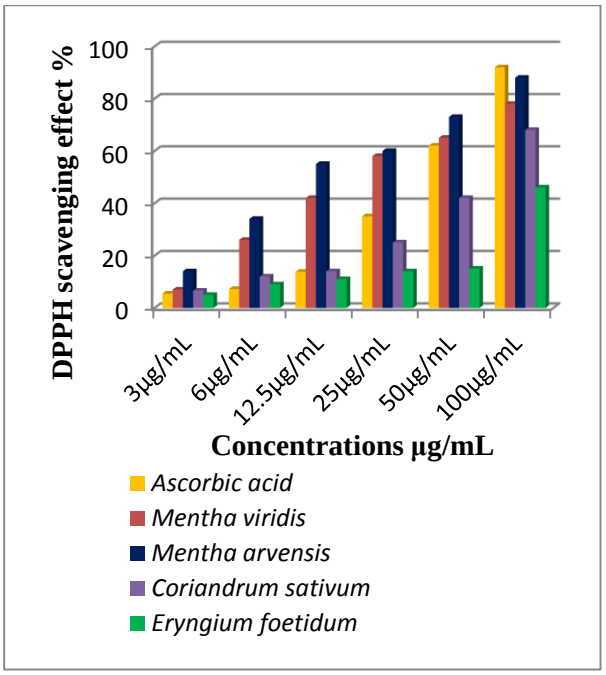

Figure 4. Evaluation of percentage of DPPH scavenging activity of leaf methanolic extract of *Mentha viridis*, *Mentha arvensis*, *Coriandrum sativum* and *Eryngium foetidum* with respect of standard ascorbic acid.

radical scavenging activity (20). Total flavonoid content (TFC) from methanolic extracts of four different plant materials were estimated in terms of rutin hydrate equivalent (RUE)/g of sample extract using the standard curve equation y=0.002x+0.034, r^2=0.988 (Figure 3) and ranged between 384.2 mg RUE/g of sample and 62 mg RUE/g of sample (Table 2). Similar to TPC, total flavonoid content

was the highest in *Mentha viridis* and the lowest in *Eryngium foetidum*. Comparing amount of TPC and TFC, lower TPC was observed and this might be due to the interference of other oxidation substrate during the Folin-Ciocaltue assay (21). On the other hand, aluminum chloride reaction to determine flavonoid contents was proved to be specific only for flavones and flavonols (22) and for this reason might gave somewhat more value than TPC.

The DPPH radical has been widely used to evaluate the free radicals scavenging ability of various natural products and has been accepted as a model compound for free radicals originating in lipids (23). In this assay, the purple chromogen radical was reduced by antioxidant/ reducing compounds to the corresponding pale yellow hydrazine. The reducing ability of antioxidants towards DPPH was evaluated by monitoring the absorbance decrease at 517 nm against various concentrations of sample extract. It means methanolic extract of plant at higher concentrations captured more free radicals formed by DPPH resulting into decrease in absorbance and increase in percentage of DPPH scavenging effect. Among the four studied materials the order of percent inhibition was *Mentha arvensis*>*Mentha viridis*>*Coriandrum sativum*>*Eryngium foetidum* (Figure 4). Inhibition capacity at 50% (IC_{50}) was calculated from linear regression equation by plotting the studied concentrations against percentage of DPPH scavenging activity. Lower the IC_{50} value higher in antioxidant capacity and therefore *Mentha arvensis* (IC_{50}=26.21±2.08 µg/mL) is the most active in percentage inhibition; even better than the standard ascorbic acid which had IC_{50} of 48.05±2.1µg/mL. Both *Mentha arvensis* and *Mentha viridis* contain high amount of TPC and TFC which is in accordance with their higher DPPH scavenging activity. Even these two materials showed better antioxidant activity than standard ascorbic acid in lower concentrations (3µg/mL - 50µg/mL), though at 100µg/mL ascorbic acid gave the highest activity among all (Figure 4). *Eryngium foetidum* showed the lowest antioxidant activity at various concentrations (3µg/mL - 100µg/mL) in line with its lower phenolic and flavonoid content. Though TPC and TFC estimated in *Mentha viridis* was found maximum, percentage of DPPH scavenging effect was second highest followed by *Mentha arvensis*. Reason might be explained as the antioxidant activity observed was not solely from the phenolic and flavonoid content, might also come from the presence of other antioxidant secondary metabolites, such as volatile oils, carotenoids, and vitamins as well as the synergistic effects among them (24a and 24b).

CONCLUSION

Present study evaluated antioxidant potential of four widely consumed aromatic herbs of Bangladesh following TPC, TFC and DPPH radical scavenging activity. Among the studied materials *Mentha viridis* showed the highest TPC and TFC though placed second highest regarding percentage DPPH scavenging activity after *Mentha arvensis*. Lowest IC_{50} value obtained in *Mentha arvensis* represent the most antioxidant efficacy. However, all the four herbs exhibited commendable antioxidant activity and thus will increase interest of mass people for naturally occurring antioxidants with consequent health benefits.

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