Bangladesh Pharmaceutical Journal **28**(1): 00-00, 2025 (January)

In vitro Antioxidant and Antibacterial Activities of Ethanolic Extract of *Mentha viridis*

Nusrat Jahan Juthy[1], Gazi Jahirul Islam[2], Abdullah Zehad[1], Mamunur Rashid[3], Md. Masud Parvez[2] and Shaheda Zannah[1]

[1]Department of Pharmacy, Southeast University, Dhaka-1208, Bangladesh
[2]Department of Chemistry, University of Barishal, Barishal-8254, Bangladesh
[3]Department of Pharmacy, Rajshahi University, Rajshahi-6205, Bangladesh

(Received: May 4, 2024; Accepted: September 25, 2024; Published (web): October 3, 2024)

Abstract

Mentha viridis has been used as folk medicine traditionally for diverse pharmacological activity and used worldwide for its aroma for different purposes in particular with tea. This research was investigated to examine the antioxidant and antibacterial activities of ethanolic extract of *M. viridis*. The antioxidant capacity was assessed by several assays namely DPPH free radical scavenging assay, total antioxidant assay, total phenolics and total flavonoids content assay. Determination of total phenolics and total flavonoids were performed spectrophotometrically using Folin-Ciocalteu reagent and aluminium chloride colorimetric assay methods respectively, while the free radical scavenging activity was estimated by DPPH (2, 2-diphenyl-2-picrylhydrazyl) method. The antibacterial activity was estimated by agar disk diffusion method against a number of bacterial strains. The results exhibited that ethanolic extract *M. viridis* is the abundant source of phenolics and flavonoids but the total antioxidant activity was detected to be the highest in comparison to other phytochemical constituents. Extract showed robust free radical scavenging activity at a concentration of 500 µg/ml. The ethanolic extract of *M. viridis* displayed moderate inhibitory activity against the microorganisms *S. aureus* and *E.coli*. The interpretation stipulates that ethanolic extract of *M. viridis* is a potential origin of natural antioxidants due to presence of mainly polyphenolic components in it.

Key words: *Mentha Viridis*, antioxidant, antibacterial, phytochemical, free radicals, DPPH.

Introduction

There is a strong link between the accumulation of free radicals and pathophysiological manifestation of innumerable threatening diseases for instance, diabetes, heart diseases, neurodegenerative disorders, atherosclerosis cancer, etc. (Gilgun-Sherki *et al.*, 2002). A free radical is any atom or molecule possessing an unpaired electron, which makes it very unstable in nature. Therefore, by way of electron pairing these free radicals have a strong desire to be stable mostly with biological macromolecules such as DNA, nucleic acids, proteins & lipids and subsequently cause protein and DNA damage (Gilgun-Sherki *et al.*, 2002; Rahman *et al.*, 2015). In living organism, reactive oxygen species (ROS) and reactive nitrogen species (RNS) which are termed as free radicals are created through the cellular redox process, which have advantageous effects on cellular responses and immune function. On the contrary, at high concentration, accumulation of these free radicals causes oxidative stress which takes part in damaging of cell structures and development of numerous chronic and degenerative disorders (Pham-Huy *et al.*, 2008).

The oxidative stress can be counterbalanced either by innate antioxidant defense mechanism or by supplying antioxidants from external sources like dietary intake and/or supplements (Valko *et al.*, 2005; Valko *et al.*, 2006). Antioxidants are called reducing agents because they donate electrons to free radicals

Corresponding author: Shaheda Zannah; Email: kazi.zannah@gmail.com
DOI: ....

and thereby stabilize them and in turn they get oxidized (Rahaman *et al.*, 2023). Besides endogenous antioxidants (glutathione peroxidase, catalase, superoxide dismutase, etc.) exogenous antioxidants also known as natural *antioxidants* (α-tocopherol, ascorbic acid, carotenoids, minerals, flavonoids, and tannins) play a major role in defense mechanism (Bhattacharyya *et al.*, 2014). Many researches have disclosed that natural antioxidants in particular phenolic compounds are enormously present in natural sources such as, plants, fruits, vegetables, herbs, cereals, oils, and spices (Alesiani *et al.*, 2010; Miliauskas *et al.*, 2004; Rahaman *et al.*, 2023).

Mentha viridis, alternatively known as *Mentha spicata* is a medicinal plant commonly called spearmint, of the Lamiaceae family, widely grown in nature (Liu *et al.*, 2012). Traditionally, this medicinal plant is utilized for the therapy of numerous illness such as, diabetes, respiratory diseases and skin disorders (Idm'hand *et al.,* 2020; Jamila & Mostafa, 2014; Salhi *et al.,* 2019). Importantly, several studies reported that chemical characterization of *M. viridis* revealed the presence of phytochemical constituents like, polyphenols, flavonoids, steroids, tannins, glycosides and triterpenes (El-Haoud *et al.*, 2018). One study reported that, ethanolic extracts of *M. viridis* contain a substantial amount of phenolic compounds including polyphenols, flavonoids and caffeic acid derivatives (Benedec *et al.*, 2013). In essential oils extracted from *M. viridis*, carvone was found as entire primary component besides trans-carveol, limonene, linalool, menthone, piperitone, piperitone oxide, isomenthone (Salehi *et al.*, 2019).

This work was aimed to study the *in vitro* antioxidant capacity by DPPH radical scavenging assay. Total flavonoids and total phenolic contents were also examined to inquire into the antioxidant capacity of phytochemicals present in *M. viridis*. Antibacterial activity of the studied plant extract was also inspected by the potential to inhibit the growth of ten bacterial strains.

Methods and materials

Chemicals: All chemicals used in the investigation were of analytical category. DPPH (2, 2-diphenyl-2-picrylhy-drazyl), Folin–Ciocalteu reagent, gallic acid (GA), ascorbic acid (AA), catechin (CA), sodium phosphate (Na_3PO_4), sodium carbonate (Na_2CO_3), aluminum chloride ($AlCl_3$) were purchased from Sigma Chemical company, USA. Sulphuric acid (H_2SO_4), hydrochloric acid (HCl), potassium acetate (CH_3COOK) were bought from Merck, Germany.

Plant collection: Leaves and barks of *M. viridis* were collected from Kawranbazar, Dhaka 1215, Bangladesh, in September 2015. The whole plant (leaves and barks) was spotted by a specialist of Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh. The accession number is DACB-41939.

Extract preparation: Leaves and barks of *M. viridis* were rigorously cleaned with water and afterwards dried for seven days under sunlight. With an appropriate grinder machine coarse powder were obtained from the plant parts after finishing the procedure. The dried and powdered materials (100 g) from each plant part were soaked in 500 ml of 90 % ethanol for the duration of 14 days at room temperature with casual shaking. Cotton filter and Whatman No. 1 filter paper were applied to filter the solution. A rotary evaporator (Bibby Sterilin Ltd, UK) was employed to concentrate the filtrate at 40 °C. A semi solid extract was obtained when the extra solvent was completely evaporated.

Methods of evaluating antioxidant activity: DPPH (2, 2-diphenyl-2-picrylhy-drazyl) radical scavenging assay: To evaluate the antioxidant activity of the extract on the stable radical DPPH, the method was followed as described by Choi's group (Choi *et al.*, 2000). 2 ml of different concentrations of ethanolic solution of plant extract (31.6 – 500 μg/ml) and 3 ml of ethanolic solution of DPPH were mixed very well. Then the reaction mixture was vortexed vigorously and permitted to rest for 30 minutes at room temperature in a dark place. The absorbance was measured at 517 nm using a spectrophotometer against a blank. Ascorbic acid was

used as a reference. The experiments were done at least three times for each concentration. The % DPPH radical scavenging activity was assessed by using the following formula:

$$\% \text{ DPPH radical scavenging activity} = \{(A_{blank} - A_{sample}) / A_{blank}\} \text{ X } 100$$

Where, A_{blank} and A_{sample} represents the absorbance of the control and the absorbance of the extract/standard respectively. The values of % of inhibition was plotted against concentration, and IC_{50} was calculated from the graph.

Determination of total phenolic contents: Total phenolic contents of ethanolic extract of *M. Viridis* was evaluated employing the method as reported previously (Rahman *et al.*, 2015). 0.5 ml of plant extract (whole plant) of various concentrations (7.8 – 125 µg/ml), 2.5 ml of 10 % Folin–Ciocalteu reagent, 2.5 ml of Na_2CO_3 (7.5 %) were withdrawn. The reaction mixture was vortexed very well and left for 20 minutes at 25 °C for the color growth. The absorbance was estimated against a blank at 765 nm employing a spectrophotometer. The standard curve was constructed using 62.5, 125, 250, 500, 1000 µgm/ml solution of gallic acid. Total phenolic values were denoted in respect of gallic acid equivalent, mg of GA/g of dry extract. The total amount of phenolic compounds in ethanolic plant extract (whole plant) and in diverse fractionates in gallic acid equivalents (GAE) was estimated by the following formula:

$$C = (c \text{ x } V)/m$$

Where C = total amount of phenol compounds, mg/g plant extract, in gallic acid equivalents (GAE, in mg). c = the gallic acid concentration which was obtained from the calibration curve in mg/ml. V = the extract volume, in ml and m = the plant extract mass, in g.

Determination of total antioxidant capacity: Total antioxidant capacity of ethanolic extract of *M. Viridis was* examined by the method of phosphomolybdenum (Prieto *et al.*, 1999). 0.5 ml of plant extract/standard at various concentrations, 3 ml of 0.6 M sulphuric acid, 28 mM sodium phosphate and 1 % ammonium molybdate were mixed and incubated at 95 °C for 90 minutes to complete the reaction. The reaction mixture was allowed to cool to room temperature. The absorbance was estimated at 695 nm using a spectrophotometer (Shimadzu, USA) against a blank.

Determination of total flavonoids: The aluminum chloride colorimetric method was utilized to find out the total flavonoid quantity of ethanolic extract of *M. viridis* as reported elsewhere *(Shraim et al.*, 2021). To render the reaction, 0.5 ml of plant extract or standard at varying concentrations, 1.5 ml of ethanol, 0.1 ml of 10 % aluminum chloride solution, 0.1 ml of 1 M potassium acetate solution, 2.8 ml of distilled water were added into the test tube and rest at room temperature for 30 minutes. By using a spectrophotometer, the absorbance was measured at 420 nm against a blank. In plant extracts, the total amount of flavonoid compounds in quercetin equivalents was computed by the following equation (Dolkun *et al.*, 2023).

$$C = (c \text{ x } V)/m$$

Where, C = total amount of flavonoid compounds, mg/g plant extract, in quercetin equivalent (QUE, in mg). c = the quercetin concentration which was obtained from the calibration curve, in mg/ml. V = the extract volume, in ml and m = the plant extract weight, in g.

Experimental details of antibacterial test

Test organisms: The antibacterial activity of plant extract of *M. viridis* was evaluated by agar disk diffusion method (Bagamboula *et al.*, 2004). This test was done with subculture bacteria, but not with pure bacteria. In order to evaluate the antibacterial activity of the plant extract a total of five gram positive and five gram negative strains of bacteria (table 4) were used. The microbiology research laboratory, Department of Pharmacy, Southeast University supplied these organisms for the experiment.

Preparation of medium: 28 g of nutrient agar media was reconstituted with 1 l distilled water (2.8 % w/v) instantly (Neetu *et al.*, 2019). Each of 5 ml media were taken in a total number of 10 test tubes to prepare plates and slants separately. The test tubes

were plugged with cotton, which was followed by the sterilization in an autoclave at 121°C for 15 minutes.

Preparation of subculture: The test organisms were moved with an inoculating loop from the pure culture to the agar slants beneath a laminar air flow unit. In order to ensure the growth of the test organisms, the inoculated slants were then incubated at 37 °C for 18 - 24 hrs.

Preparation of test plates: With the aid of an inoculating loop, test organisms were transported from the subculture to a test tube which, contained 20 ml autoclaved medium and subsequently test tube was shaken to obtain a static suspension of the organism. It was straight away transferred to a sterile Petri dish and was revolved both clockwise and anticlockwise in a systematic manner to ensure homogeneous dispersion of organisms. All the plates were prepared for each of the organisms following the same procedure and afterwards stored at 4 °C after cooling to room temperature.

Preparation of disk: Two kinds of disks were used for antibacterial activity test. Sample disks: Sterile filter paper disks (5 mm in diameter) were placed in a blank Petri dish. Sample solution of desired concentration was put in the filter paper disks using a micropipette and allowed to leave for a few minutes in an aseptic condition for the total removal of solvent. Blank disks: Only pure ethanol was used as negative control.

Preparation of disk containing sample: The process was conducted following a method as narrated by (Srikacha & Ratananikom, 2020). Individual 20 sterile disks (5 for gram positive and 5 for gram negative bacteria, and 10 for control), each 5 mm in diameter, were placed on a nutrient agar plate that had been inoculated with 100 µl of the bacterial strain. 500 µg/disc of crude extract were put onto the sterile blank disk. The plates were inverted afterwards and incubated at 37 °C for 24 hours. Kanamycin was employed as positive control. The antibacterial activities of the test materials and standard antibiotic disk were estimated by calculating the diameter of the zones of inhibition in millimeters using a Vernier caliper.

Statistical analysis: All experiments were performed at least three times. The statistical analysis was carried out by one-way analysis of variance (ANOVA), followed by Dunnett's post-hoc test or students paired or unpaired t-test where applicable. The outcomes were displayed as mean ± SEM.

Results and Discussion

Determination of DPPH radical scavenging activity: The free radical scavenging activity of the extract of *M. viridis* was evaluated by DPPH radical scavenging assay as described in the experimental section. Figure 1 & figure 2 show the % of scavenging activity and IC_{50} values of standard ascorbic acid (AA) and ehanolic extracts of *M. viridis.* It can be found that, different concentrations of ethanolic extracts of *M. viridis* showed different radical scavenging activity with IC_{50} value of (27.73 ± 0.42) µg/ml. As can be found from figure 1 that, AA showed approximately 97.19 ± 0.35 % radical scavenging activity at the concentration of 500 µg/ml but in case of ethanolic extracts it was noticed that with increasing concentration scavenging activity increased dramatically and reached to approximately 91 ± 0.63 % when the extract concentration was 500 µg/ml. The IC_{50} value of extract and AA are 16.29 ± 0.39 and 27.73 ± 0.42 respectively (Figure 2). This result suggests that extract of *M. viridis* contains a substantial free radical scavenging activity when compared with reference AA.

Estimation of total phenolic contents: Total phenolic contents of the sample was assessed in relation to the standard curve equation for gallic acid (GA) (standard curve equation: 0.0064x + 0.055, R^2 = 0.9991) as shown in figure 3. The result was shown in terms of gallic acid equivalent (GAE), (mg of GA/g of dried extract). Different concentrations of GA were estimated but a single concentration of extract was chosen to compare comfortably which is displayed in table 1. It was found that the absorbance of GA and extract at the concentration of 500 µg/ml was 3.231 and 0.494 respectively. The total phenolic quantity was detected in extract 73.16 mg of GA/g of dried extract.

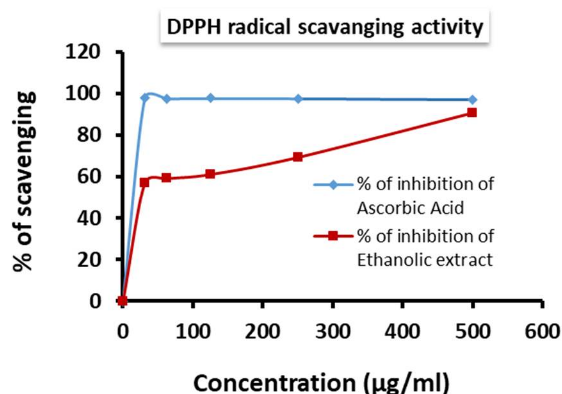

Figure 1. Standard and sample curve of DPPH radical scavenging activity.

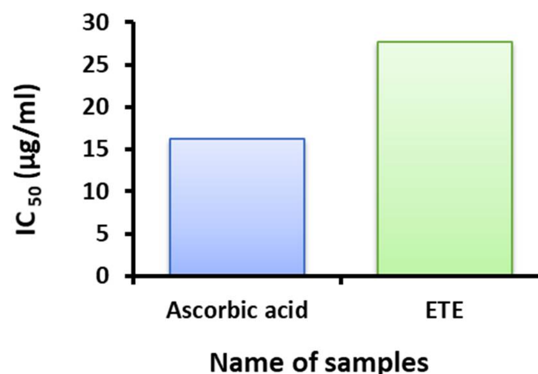

Figure 2. IC_{50} of *M. viridis* and ascorbic acid. Where, ETE = Ethanolic extract.

Determination of total antioxidant capacity: The total antioxidant capacity of the ethanolic extract was evaluated by phosphomolybdenum method. The phosphomolybdenum method relies on the reduction of Mo (VI) to Mo (V) by samples/standard and subsequent generation of phosphate/Mo (V) complex which is green in color with a maximal absorption at 695 nm. Catechin (CA) was utilized as standard. The result was figured out from the calibration plot of CA (Y = 0.001x + 0.0662, R^2 = 0.9877). Total antioxidant capacity of CA (standard) and plant extract were depicted below in table 2 and figure 4 respectively. Increased absorbance of CA with increasing concentration indicates higher antioxidant capacity as can be seen from table 2. At the concentration of 1000 µg/ml the absorbance of CA and extract was 1.046 and 0.436 respectively. The extract was found to contain total antioxidant 370 mg of CA/g of dried extract. It can be found that, ethanolic extract of *M. viridis* possessed a good antioxidant capacity in comparison with CA.

Determination of total flavonoid contents: The result of total flavonoid contents of the sample was shown as mg of quercetin acid equivalent (QE)/g of dried extract. The total flavonoid contents of extract was computed from the calibration plot of quercetin (Y = 0.0136x – 0.0069, R^2 = 0.9994). The total flavonoid contents of quercetin at diverse concentrations (7.8 – 125 µg/ml) and single concentration of extract are exhibited in table 3 and a standard curve for total flavonoid contents is given in figure 5. The absorbance of quercetin and extract at 125 µg/ml was 1.69 and 0.89 respectively as shown in table 3. The total flavonoid content found in extract was 78.15 mg quercetin acid equivalent (QE)/g of dried extract. This result suggests that extract of *M. viridis* contains substantial total flavonoid content.

Table 1. Absorbance of GA at different concentrations and extract at single concentration.

Name of standard	Concentration (µg/ml)	Absorbance
GA	3.906	0.009
	7.8125	0.116
	15.625	0.160
	31.25	0.271
	62.5	0.458
	125	0.848
	250	1.715
	500	3.231
Extract	500	0.494

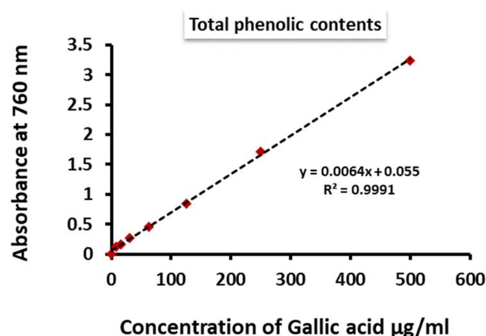

Figure 3. Standard curve for total phenolics.

Table 2. Absorbance of CA at different concentrations and extract at single concentration.

Name of standard	Concentration (μg/ml)	Absorbance
Catechin	15.63	0.076
	31.25	0.108
	62.50	0.195
	125	0.213
	250	0.443
	500	0.529
	1000	1.046
Extract	1000	0.436

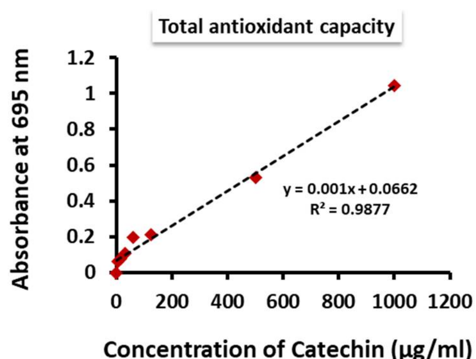

Figure 4. Standard curve for total antioxidant capacity.

Assessment of antibacterial activity of plant extract: The ethanolic extract of *M. viridis* was investigated to evaluate its antibacterial activity in opposition to a number of five gram positive and five gram negative bacteria using disk diffusion method. Standard antibiotic disks of kanamycin utilized for bacterial species was 30 μg/disk. Antibacterial screening of extract of *M. viridis* was applied at a concentration of 500 μg/disk. The evaluation of antibacterial activity of extract opposed to a number of gram positive and gram negative bacteria are presented in table 4. It can be seen that extract was only potentially effective against *S. aureus* and *E. coli*.

Table 3. Absorbance of quercetin at different concentrations and extract at single concentration.

Name of standard /sample	Concentration (μg/ml)	Absorbance
Quercetin	7.81	0.108
	15.63	0.189
	31.25	0.403
	62.50	0.869
	125	1.69
Sample	125	0.89

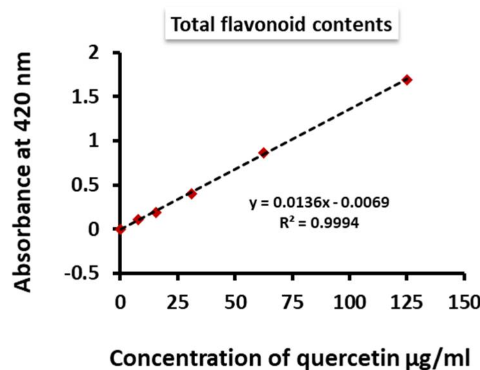

Figure 5. Standard curve for total flavonoid contents.

Table 4. Antimicrobial effect of ethanolic plant extract of *M. viridis* on five gram positive and gram negative bacteria, compared to that of positive standard (kanamycin). [a]Each value represents the average of three analyses ± standard deviation.

	Diameter of zone of inhibition (mm)	
Bacterial strain	**Extract[a]**	**Kanamycin[a]**
Gram positive		
Staphylococus aureus	12 ± 1.73	32 ± 1.0
Bacillus megaterium	-	24 ± 0.5
Bacillus subtilis	-	26 ± 1.0
Sarcina lutea	-	24 ± 1.0
Bacillus cereus	-	32 ± 2.0
Gram negative		
Escherichia coli	9 ± 0.50	32 ± 1.0
Vibrio mimicus	-	30 ± 2.3
Vibrio parahemolyticus	-	30 ± 1.3
Sheigella boydii	-	26 ± 0.5
Pseudomonouss aeruginosa	-	26 ± 1.0

DPPH radical scavenging activity: Free radical scavenging by DPPH assay is a quick, straightforward and economical method for the screening of antioxidant capacity of plant extracts which can donate hydrogen and thus behave as a free radical scavenger (Nanjo *et al.*, 1996). In this method, DPPH, a stable free radical, is eliminated by the addition of antioxidant present in plant extract in a concentration dependent mode. Usually, DPPH solution is a violet colored solution and once it's get reduced by antioxidants present in plant extract DPPH solution turns to yellow colored product, diphenylpicryl hydrazine. The extent of color change is linearly proportional to the concentration and antioxidant capacity of extract (Krishnaiah *et al.*, 2011). In this study, ethanolic extract of *M. viridis* showed considerable free radical scavenging activity when compared to standard AA. Maximum scavenging activity found at concentration of 500 µg/ml with 91 ± 0.63 % reducing capacity, which is nearly closer to reference as represented in figure 1. According to previously reported studies, phytochemicals such as polyphenols and tocopherols can fight DPPH free radicals by their capability to give hydrogen (Huang *et al.*, 2005). Besides, a highly significant positive correlation is observed between polyphenolic contents and DPPH free radical scavenging activity with IC_{50} value of 27.73 ± 0.42 µg/ml (Figure 2) (Huang *et al.*, 2005). This result suggests that *M. viridis* contains phytochemicals in particular polyphenols, which have the capability to give hydrogen or transfer electrons to a free radical and consequently converting them into non-toxic species and thereby cease the potential damage.

Total phenolic contents: Phenolic constituents are considered as secondary metabolites in plants and are familiar for the usefulness in a wide variety of therapeutic uses such as free radical scavenging activities, antioxidant, anticarcinogenic, reduction in cardiovascular complications, etc. (Umamaheswari & Chatterjee, 2008). Phenolic compounds are pondered as essential constituents for the plants and their scavenging activity is predominantly ascribed to the manifestation of one or more hydroxyl groups in the aromatic ring (Naczk & Shahidi, 2004). The method of estimating total phenolic contents of plant extracts by Folin–Ciocalteu reagent represents simplicity, reproducibility and suitability (Huang *et al.*, 2005). The findings in this study demonstrated that, the ethanolic extract *M. viridis* contains a significant amount of phenolic constituents that is 73.16 mg of GA/g of dried extract. This value proves that *M. viridis* is the richest source of phenolics, which might contribute to the antioxidant properties of this plant. Additionally, a linear regression coefficient value R^2 = 0.9991 observed between the total phenolics and free radical scavenging capacity.

Total flavonoid contents: Flavonoids are a group of polyphenols naturally occurring in plants and according to previously reported studies flavonoids have numerous biological activities such as, antiallergic, antibacterial, antiviral, anti-inflammatory, anticancer, antihepatotoxic etc. (Di Carlo *et al.*, 1999; Montoro *et al.*, 2005). Flavonoids are very effective in scavenging the reactive oxygen species for instance, singlet oxygen and numerous free radicals liable for a variety of diseases (Cao *et al.*, 1997). In this work, the extract was found to contain 78.15 mg quercetin acid equivalent (QE)/g of dried extract, which seems quite substantial in flavonoids quantity. The observed regression coefficient value R^2 = 0.994 correlates positively between the total flavonoid contents and the total antioxidant capacity. This result recommends that *M. viridis* extract has strong antioxidant potentiality in neutralizing the free radicals due to having significant quantity of total phenolics and total flavonoids.

Total antioxidant capacity: The total antioxidant capacity of plant extract was measured spectrophotometrically by utilizing the method of phosphomolybdenum, which relies on the reduction of Mo (VI) to Mo (V) by antioxidants present in fractions and in consequence forms a green colored phosphate/Mo (V) complex. This current study exhibits that ethanolic extract of *M. viridis* represented maximum reducing capacity that is 370 mg of CA/g of dried extract. Many studies suggested that, phytochemicals present in medicinal plants particularly polyphenols and flavonoids contribute to

phosphomolybdate reduction or scavenging activity to a large extent (Khan *et al.*, 2012; Sharififar *et al.*, 2009). This result suggests that the strong antioxidant capacity of plant extract is primarily owing to the existence of polyphenol contents (Falleh *et al.*, 2008). Moreover, a strong positive correlation (regression coefficient, R^2 = 0.9877) observed in between the antioxidant capacity and polyphenol contents. Therefore, it can be concluded that, *M. viridis* plant extract possesses significant free radical scavenging activity as a consequence of occurrence of phenolic constituents available in the plant extract (Falleh *et al.*, 2008).

Antibacterial activity: The antibacterial activity of plant extract was tested against five gram positive and five gram negative bacteria. The extract was found to potent against only one gram positive and one gram negative bacteria namely *S. aureus* and *E. coli* respectively. At concentration of 500 µg/disk the ethanol extract showed moderate antibacterial activity with the zones of inhibitions of 12 ± 1.73 and 9 ± 0.50 mm respectively when compared with the reference kanamycin. On the contrary, four gram positive bacterial strain viz. *B. megaterium*, *B. subtilis*, *S. lutea*, *B. cereus* and four gram negative bacterial strain viz. *V. mimicu, V. parahemolyticu, S. boydi, P. aeruginosa* used in this study showed no susceptibility at the concentration of plant extract utilized in this study. In accordance with previously reported studies, some phytochemicals for instance terpenoids, alkaloids and polyphenols have antimicrobial efficiency and are responsible for either the microbial cell membrane disruption or inhibition of respective enzymes which are required for amino acids biosynthesis (Burt, 2004; Gill & Holley, 2006). Some other researchers suggested that the observed antimicrobial activities of plant extract were supposed to the hydrophilic nature of extract, which allow them to react with microbial protein and mitochondria. Consequently, permeability changed and microbial structure disrupted (Friedman *et al.*, 2004; Tiwari *et al.*, 2009). Therefore, the inhibitory effect found in the extract was ascribed to the manifestation of valuable phytochemical constituents (polyphenols, flavonoids and terpenoids, etc.).

Conclusion

In order to assess the *in vitro* antioxidant activity of ethanolic extract of *M. viridis* different assay methods were explored. The extract contains considerable DPPH free radical scavenging capacity, which was comparable to reference used in this study. The direct positive correlations between phenolics (total phenols, total flavonoids and total antioxidant) and free radical scavenging capacity were highly significant denoting that polyphenols might play a key role in free radical quenching activity. The studied extract displayed the highest amount of phosphomolybdate reducing capacity having 370 mg of CA/g of dried extract. *M. viridis* extract also exhibited antibacterial activity to some extent against *S. aureus* and *E. coli* in comparison to kanamycin. All the aforementioned findings recommend that *M. viridis* contains significantly higher amount of natural antioxidants which possibly will assist in preventing the advancement of any disease because of free radicals such as cardiovascular diseases, inflammatory diseases or cancer and can also be useful in a variety of health conditions. However, further investigations on *M. viridis* will be required to isolate and identify the phytochemical constituents in particular the antioxidant components for the medical purpose.

Conflict of interest

All authors announce that there is no conflict of interest with respect to publication of this manuscript.

Acknowledgement

Authors are thankful to Bangladesh National Herbarium for identifying the experimental plants.

References

Alesiani, D., Canini, A., D'Abrosca, B., DellaGreca, M., Fiorentino, A., Mastellone, C., Monaco, P. and Pacifico, S. 2010. Antioxidant and antiproliferative activities of phytochemicals from Quince (*Cydonia vulgaris*) peels. *Food Chem.* **118**, 199-207.

Bagamboula, C., Uyttendaele, M. and Debevere, J. 2004. Inhibitory effect of thyme and basil essential oils, carvacrol, thymol, estragol, linalool and p-cymene towards *Shigella sonnei* and *S. flexneri*. *Food Microbiol.* **21**, 33-42.

Benedec, D., Vlase, L., Oniga, I., Mot, A. C., Silaghi-Dumitrescu, R., Hanganu, D., Tiperciuc, B. and Crişan, G. 2013. LC-MS analysis and antioxidant activity of phenolic compounds from two indigenous species of *Mentha*. Note I. *Farmacia*. **61**, 262-267.

Bhattacharyya, A., Chattopadhyay, R., Mitra, S. and Crowe, S. E. 2014. Oxidative stress: an essential factor in the pathogenesis of gastrointestinal mucosal diseases. *Physiol. Rev.* **94**, 329-354.

Burt, S. 2004. Essential oils: their antibacterial properties and potential applications in foods: a review. *Int.J. Food Microbiol.* **94**, 223-253.

Cao, G., Sofic, E. and Prior, R. L. 1997. Antioxidant and prooxidant behavior of flavonoids: structure-activity relationships. *Free Radic. Biol. Med.* **22**, 749-760.

Choi, H. Y., Jhun, E. J., Lim, B. O., Chung, I. M., Kyung, S. H. and Park, D. K. 2000. Application of flow injection—chemiluminescence to the study of radical scavenging activity in plants. *Phytother. Res.* **14**, 250-253.

Di Carlo, G., Mascolo, N., Izzo, A. A. and Capasso, F. 1999. Flavonoids: old and new aspects of a class of natural therapeutic drugs. *Life Sci.* **65**, 337-353.

Dolkun, A., Aimaiti, Z., Muhammad, T., Guo, G. and Reheman, A. 2023. FIA Online spectrophotometric determination of total flavonoids in plants and its application for batch adsorption kinetics. *Ind. Eng. Chem. Res.* **62**, 14224-14233.

El-Haoud, H., Boufellous, M., Berrani, A., Tazougart, H. and Bengueddour, R. 2018. Screening phytochimique d'une plante medicinale: *Mentha spicata* L. *Am. J. Innov. Res. Appl. Sci.* **7**, 226-233.

Falleh, H., Ksouri, R., Chaieb, K., Karray-Bouraoui, N., Trabelsi, N., Boulaaba, M. and Abdelly, C. 2008. Phenolic composition of *Cynara cardunculus* L. organ and their biological activities. *Comptes Rendus. Biologies* **331**, 372-379.

Friedman, M., Henika, P. R., Levin, C. E. and Mandrell, R. E. 2004. Antibacterial activities of plant essential oils and their components against *Escherichia coli* O157: H7 and *Salmonella enterica* in apple juice. *J. Agri. Food Chem.* **52**, 6042-6048.

Gilgun-Sherki, Y., Rosenbaum, Z., Melamed, E. and Offen, D. 2002. Antioxidant therapy in acute central nervous system injury: current state. *Pharmacol. Rev.* **54**, 271-284.

Gill, A. and Holley, R. 2006. Disruption of *Escherichia coli*, *Listeria monocytogenes* and *Lactobacillus sakei* cellular membranes by plant oil aromatics. *Int. J. Food Microbiol.* **108**, 1-9.

Huang, D., Ou, B. and Prior, R. L. 2005. The chemistry behind antioxidant capacity assays. *J. Agri. Food Chem.* **53**, 1841-1856.

Idm'hand, E., Msanda, F. and Cherifi, K. 2020. Ethnopharmacological review of medicinal plants used to manage diabetes in Morocco. *Clin. Phytosci.* **6**, 1-32.

Jamila, F. and Mostafa, E. 2014. Ethnobotanical survey of medicinal plants used by people in Oriental Morocco to manage various ailments. *J. Ethnopharmacol.* **154**, 76-87.

Khan, R. A., Khan, M. R., Sahreen, S. and Ahmed, M. 2012. Assessment of flavonoids contents and *in vitro* antioxidant activity of *Launaea procumbens*. *Chem. Cent. J.* **6**, 1-11.

Krishnaiah, D., Sarbatly, R. and Nithyanandam, R. 2011. A review of the antioxidant potential of medicinal plant species. *Food Bioprod. Process* **89**, 217-233.

Liu, K. H., Zhu, Q., Zhang, J. J., Xu, J. F. and Wang, X. C. 2012. Chemical composition and biological activities of the essential oil of *Mentha spicata* Lamiaceae. *Adv. Mater. Res.* **524**, 2269-2272.

Miliauskas, G., Venskutonis, P. and Van Beek, T. 2004. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. *Food Chem.* **85**, 231-237.

Montoro, P., Braca, A., Pizza, C. and De Tommasi, N. 2005. Structure–antioxidant activity relationship of flavonoids isolated from different plant species. *Food Chem.* **92**, 349-355.

Naczk, M. and Shahidi, F. 2004. Extraction and analysis of phenolics in food. *J. Chromatogr. A.* **1054**, 95-111.

Nanjo, F., Goto, K., Seto, R., Suzuki, M., Sakai, M. and Hara, Y. 1996. Scavenging effects of tea catechins and their derivatives on 1, 1-diphenyl-2-picrylhydrazyl radical. *Free Radi. Biol. Med.* **21**, 895-902.

Neetu, K., Khan, M. A., Sharma, A. K. and Besra, S. 2019. Freshwater edible crab, sartoriana spinigera as a source of antibacterial agent against clinical pathogens. *Adv. Innov. Res.* 70.

Pham-Huy, L. A., He, H. and Pham-Huy, C. 2008. Free radicals, antioxidants in disease and health. *Int. J. Biomed. Sci.: IJBS.* **4**, 89.

Prieto, P., Pineda, M. and Aguilar, M. 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Anal. Biochem.* **269**, 337-341.

Rahaman, M. M., Hossain, R., Herrera-Bravo, J., Islam, M. T., Atolani, O., Adeyemi, O. S., Owolodun, O. A., Kambizi, L., Daştan, S. D. and Calina, D. 2023. Natural antioxidants from some fruits, seeds, foods, natural products and associated health benefits: an update. *Food Sci. Nutr.* **11**, 1657-1670.

Rahman, M. M., Islam, M. B., Biswas, M. and Khurshid Alam, A. 2015. *In vitro* antioxidant and free radical scavenging activity of different parts of *Tabebuia pallida* growing in Bangladesh. *BMC Res. Notes.* **8**, 1-9.

Bahare, S., Ata, A., Anil Kumar, V. N., Farukh, S., Karina, R. A., Ana, R. O., Seyed, A. A., Patrick, V. T. F., Farzad, K.. and Zainul, A. Z. 2019. Antidiabetic potential of medicinal plants and their active components. *Biomolecules* **9**, 551.

Salhi, N., Bouyahya, A., Fettach, S., Zellou, A. and Cherrah, Y. 2019. Ethnopharmacological study of medicinal plants used in the treatment of skin burns in occidental Morocco (area of Rabat). *S. Afr. J. Bot.* **121**, 128-142.

Sharififar, F., Dehghn-Nudeh, G. and Mirtajaldini, M. 2009. Major flavonoids with antioxidant activity from *Teucrium polium* L. *Food Chem.* **112**, 885-888.

Shraim, A. M., Ahmed, T. A., Rahman, M. M. and Hijji, Y. M. 2021. Determination of total flavonoid content by aluminum chloride assay: a critical evaluation. *Food Sci. Tech.* **150**, 111932.

Srikacha, N. and Ratananikom, K. 2020. Antibacterial activity of plant extracts in different solvents against pathogenic bacteria: an *in vitro* experiment. *J. Acute Dis.* **9**, 223-226.

Tiwari, B. K., Valdramidis, V. P., O'Donnell, C. P., Muthukumarappan, K., Bourke, P. and Cullen, P. 2009. Application of natural antimicrobials for food preservation. *J. Agri. Food Chem.* **57**, 5987-6000.

Umamaheswari, M. and Chatterjee, T. 2008. *In vitro* antioxidant activities of the fractions of *Coccinia grandis* L. leaf extract. *Afr. J. Trad. Comple. Alter. Med.* **5**, 61-73.

Valko, M., Morris, H. and Cronin, M. 2005. Metals, toxicity and oxidative stress. *Curr. Med. Chem.* **12**, 1161-1208.

Valko, M., Rhodes, C., Moncol, J., Izakovic, M. and Mazur, M. 2006. Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chem. Biol. Interact.* **160**, 1-40.