Full Length Research Paper

Phytochemical analysis, antioxidant and antidiarrhoeal activities of methanol extract of *Fimbristylis miliacea* (L.) Vahl

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The present study determined phytochemical content, antioxidant and anti diarrhoeal effect of methanol extract of *Fimbristylis miliacea*. Phytochemical screening showed the presence of flavonoids, saponins, tannins, phenols, alkaloids and cardiac glycosides. Total phenolic, flavonoids, flavonols, tannins, β-carotene, lycopene, chlorophyll-α, and chlorophyll-β contents were found 154.13 mg GAE/g, 83.14 mg QE/g, 126.45 mg QE/g, 215.72 mg GAE/g, 1.51 µg/g, 0.66 µg/g, 7.10 mg/g and 4.25 mg/g, respectively. Antioxidant potential was compared against ascorbic acid. IC₅₀ and EC₅₀ values were determined as indices of antioxidant potential using five experiments; IC₅₀ in 2,2-diphenyl-1-picrylhydrazyl (DPPH) and hydroxyl radical scavenging assay were 138.83 and 353.82 µg/ml respectively. EC₅₀ of thiobarbituric acid assay, FRAP assay and reducing power assay were observed 2159.5, 31.59 and 129.56 µg/ml respectively. Total antioxidant capacity was 155.35 mg/g AAE. Significant reduction (p≤0.001) in fecal movement was produced by plant extract and the effect was stronger than that of the standard drug loperamide (5 mg/kg.bw). Loperamide showed 58.16% inhibition whereas extract at 400 mg/kg bw dose inhibited 80.85% diarrhea.

**Key words:** *Fimbristylis miliacea*, phytochemical content, antioxidant, antidiarrhoeal

INTRODUCTION

Indigenous plants having putative medicinal properties occupy an important place in health care system despite revolutionary advancement in synthetic drug discovery. Herbal medicines which principally use indigenous plants are very effective against various diseases and it is reported that around three-fourth population of developing countries rely on traditional medicines for primary healthcare and annual turnover in global trade in medicinal herbs is over hundred billions US dollars (Sofowora et al., 2013). Plants blessed with diverse phytochemicals are a source of myriad compounds beneficial for mankind and researchers around the world are working in pursuit to discover potential lead compounds from natural sources. *Fimbristylis miliacea* (L.) Vahl, a grass-like herb belongs to Cyperaceae family. *F. miliacea* (L.) Vahl is the medicinal herbs is over hundred billions US dollars (Sofowora et al., 2013). Plants blessed with diverse

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Fimbristylis miliacea (L.) Vahl, a grass-like herb belongs to Cyperaceae family. F. miliacea (L.) Vahl is the most preferred scientific name but in some countries, it is also named as Fimbristylis quinquangularis (Vahl) Kunth. The grass usually grows in paddy fields, shallow water along ditches, and is widely seen in countries of South and South-east Asia and Australia (Waterhouse, 1994). Among three hundred species of the genus Fimbristylis, few species are used in herbal medicine. For example, F. miliacea in fever, F. squarrosa for sore throat, F. falcata in dysentery, F. ovata in rheumatism, F. umbellaris in splenomegaly, F. pauciflora to induce labour, etc. (Simpson and Inglis, 2001). Methanol extract of F. miliacea has been found to have potent antipyretic and antiinflammatory potential (Roy et al., 2019) and moderate hypoglycemic effect (Mia et al., 2019) in mice model.

All essential cell activities are run by the energy produced by metabolism of foods principally by oxidation reaction. Generation of this vital energy always come up with free radicals such as hydroxyl (•OH), peroxide (ROO•), superoxide (O2•-)—commonly termed as reactive oxygen species (ROS) and imparts oxidative stress on the cells. These free radicals react with other biomolecules and cause severe damage to cells. Cancer (Prasad et al., 2017), aging (Desjardins et al., 2017), diabetes (Ni et al., 2016), and neurodegenerative diseases (Hung et al., 2018) are found linked with oxidative stress of reactive oxygen species. An intracellular redox homeostasis is always maintained by endogenous enzyme systems such as superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glutathione-S-transferase, etc. to keep low level of reactive oxygen species. In case of attenuated activity of endogenous molecules, antioxidants from beneficial foods and supplements are recommended to combat these intracellular oxidative damages. Exploration and identification of beneficial compounds from plants thus become important in recent years and species of different plant families have been reported with diverse therapeutic potentials like antioxidant, antinociceptive, anti-inflammatory, antipyretic, antimicrobial, anti diarrheal, cytotoxic, hepatoprotective, anti diabetic effects. The present study was aimed to investigate antioxidant and anti diarrheal potentials of methanol extract of F. miliacea as well as analyse phytochemical content of the plant.

MATERIALS AND METHODS

Preparation of plant extract

Aerial part of F. miliacea was collected from Manikgonj, Bangladesh. The species was first identified by Bangladesh National Herbarium and a specimen (No.: DACB-46517) was deposited there. The collected plant mass was washed with water, dried for ten days and then pulverized into powder with grinder. About 500 g of the powder was taken in a flat bottom flask and soaked in 80% 1500-mL methanol at room temperature for two weeks; the flask was occasionally shaken and stirred for proper mixing. The mixture was then filtered (Whatman filter paper No. 1). The filtrate collected was evaporated at 38°C and 85 rpm with rotary evaporator (RE-EV311-V, LabTech). A gummy concentrate was produced; this concentrate was our desired crude methanol extract.

Chemicals and standard drug

All reagents were of analytical grade. 2,2-diphenyl-1-picrylhydrazyl (DPPH), tannic acid, quercetin, ascorbic acid, ferric chloride, aluminium chloride, sodium carbonate, sodium acetate, potassium acetate, sodium phosphate, ammonium molybdate, methanol, hydrochloric acid, sodium hydroxide, potassium ferricyanide, ferrous sulphate, thiobarbituric acid, trichloroacetic acid, hydrogen peroxide hexane and acetone were purchased from local agent of Merck, Germany.

Qualitative phytochemical analysis

Qualitative analyses of major phytoconstituents in methanol extract of F. miliacea were carried out using standard procedures as described (Evans, 2009).

Quantitative phytochemical analysis

Total phenolic content (TPC)

Folin-Ciocalteu (FC) reagent was used to determine total phenolic content (Ebrahimizadeh et al., 2008). A calibration curve was first formulated using different concentrations of gallic acid (0-300 µg/mL). Here, about 0.5 mL of extract (1 mg/ml) was mixed thoroughly with 2.5 mL of FC reagent followed by addition of 2 mL of sodium carbonate. The mixture was then incubated 20 min at room temperature. Absorbance was then measured at 760 nm and phenolic content was expressed in mg gallic acid equivalent per g of plant extract.

Total flavonoid and flavonol content

In this experiment, quercetin was used as standard forming its curved with different concentrations and thus both flavonoid and flavonol content are generally expressed as µg quercetin equivalent (QE)/mg of extract. For determination of flavonoid, a mixture composed of 0.5 mL (1 mg/ml) extract, 1.5 mL of methanol, 0.1% of 10% aluminium chloride, 0.1 mL of potassium acetate and 2.8 mL of distilled water was prepared and then incubated for 40 min at room temperature. Absorbance of mixture was measured at 415 nm. In case of flavonol, absorbance was measured at 400 nm of the mixture of 2 mL of extract, 6 mL of 5% sodium acetate and 2 mL of 2% aluminium chloride (Rezaei-Zadeh et al., 2011).

Tannin content

Tannin content of the extract was measured by Folin-Ciocalteu reagent (Mohammed and Manan, 2015). 0.1 mL of FC reagent was mixed with 0.5 mL of extract (as 1:200 dilution) and incubated for 15 min at room temperature. 2.5 mL of 20% sodium carbonate (20%)
was then added and again incubated for 30 min. Absorbance was measured at 760 nm. Tannin content was expressed in mg gallic acid equivalent per g of extract.

Chlorophyll-α and chlorophyll-β

For determination of chlorophylls, absorbances of plant extract sample (1 mg/ml) were measured at two different wavelengths- 653 nm and 666 nm. Readings of absorbance were then put in following equations (Lichtenenthaler and Wellburn, 1983):

Chlorophyll-α (mg/ml) = 15.65A666 - 7.340A653
Chlorophyll-β (mg/ml) = 27.0A653 - 11.21A666

β-carotene and lycopene

Samples were prepared with 100 mg dried methanol extract and 10 ml of acetone-hexane (4:6) and then filtered. Absorbances were recorded at 453, 505 and 663 nm and used in the following equations:

β-carotene (mg/100 ml) = 0.216A453 - 0.304A505 + 0.452A653
Lycopene (mg/100 ml) = 0.0458A453 + 0.372A505 - 0.805A653

Results were expressed as μg of β-carotene and lycopene per g of extract.

In vitro antioxidant assays

Total antioxidant capacity

Determination of total antioxidant activity was based on the change of oxidation state of molybdenum from three to five by forming green phosphomolybdate at acidic pH 3.4 (Rice-Evans et al., 1995). At first, a reagent mixture was prepared by mixing different volumes of 0.6 M of sulphuric acid, 28 mM of sodium phosphate and 4 mM of ammonium molybdate. This reagent was then mixed with 1 ml of plant extract followed by heating at 90°C for 90 min. The mixture was then cooled to room temperature and finally absorbance was measured at 695 nm against blank. Here, ascorbic acid was used as standard. Antioxidant capacity was expressed in μg ascorbic acid per g of sample.

Hydroxyl radical scavenging assay (HRSA)

1 ml of extract of different concentrations was mixed with 250 μl of 6 mM FeSO4 and 0.5 ml of 6 mM H2O2. This mixture was gently shaken. 1 ml of 6 mM salicylic acid was then added and incubated for 30 min at room temperature (Al-Trad et al., 2018). Absorbance of the mixture was measured at 510 nm and ascorbic acid was used as standard.

Thiobarbituric acid (TBA) method

Reagent for TBA assay was prepared by adding 1 ml of 20% aqueous trichloroacetic acid with 2 ml of 0.67% aqueous thiobarbituric acid. 2 ml of plant extract was then mixed with this reagent (Mackeen et al., 2000). The mixture was subjected to boiling for 10 min. Upon cooling, the sample was centrifuged at 3000 rpm for 30 min. Absorbance of the supernatant was measured at 532 nm.

Determination of IC50 and EC50 value

IC50 (inhibition concentration) value represents concentration of antioxidant necessary to reduce initial concentration by 50%. Low IC50 indicates high antioxidant activity. EC50 (effective concentration) indicates concentration of a compound producing 50% of its maximal effect. Both values were calculated from extrapolation of graph of ‘activity versus extract concentration’. All experiments were conducted in triplicate.

Antidiarrhoeal activity in mice model

Prior approval regarding use of animal in in vivo test was taken from Departmental Research Committee. Male Swiss albino mice (age: 5-6 weeks old, weight: 20-25 g) were procured from ICDDR,B, Bangladesh. Mice were allowed to acclimatize with new environment for one week and kept in polycarbonate cage; temperature of the vivarium was maintained at 23 ± 1°C and humidity was at 55-60%. Animals were divided into five groups; each group consisted of six mice. First group was named as control group receiving only saline (10 ml/kg,bw); mice group receiving antidiarrheal drug- loperamide (5 mg/kg,bw) was called standard group. Three different concentrations of plant extract were 100, 200 and 400 mg/kg,bw and designated as FM100, FM200 and FM400.
Diarrhea was induced by oral administration of castor oil (0.2 ml/animal); while control, loperamide or plant extracts were ingested 30 min prior to castor oil intake. Immediately after castor oil administration, each mouse was placed on blotting paper and observed for 5 h. The following parameters were monitored: time to initial evacuation, evacuation classification: 1 (solid stool), 2 (semi-solid stool), and 3 (liquid stool) and evacuation index (EI). EI value was calculated according to the following formula: EI=1 x (No 1. stool) +2 x (No 2. stool) +3 x (No 3. stool). Percentage inhibition of diarrhea was calculated as (EI of vehicle -EI of sample) x 100/(EI of vehicle) (Mbagwu and Adeyemi, 2008).

Quantitative phytochemical contents

Figure 1 shows the amount of phenolic, flavonoid, flavonol, tannin, chlorophyll-α, chlorophyll-β, β-carotene and lycopene.

RESULTS

Qualitative phytochemical contents

Methanol extract of *F. miliacea* showed the presence of flavonoids, saponins, tannins, phenols, alkaloids, cardiac glycosides while anthraquinone glycosides, steroids, carbohydrates, gums, phytosterols and terpenes were not observed.

Antioxidant activity of plant extract

**Total antioxidant capacity**

Total antioxidant capacity of the extract was found as 155.35 ± 0.006 mg ascorbic acid equivalent/g extract.
Figure 2. Percentage inhibitions and absorbances of different antioxidant assays. Standard error of mean/standard deviations were determined but these statistical parameters were so small that eventually merged into the sample data point.

and was calculated from straight line equation \( y = 0.007x - 0.0401, R^2 = 0.9983 \). Here, absorbance of sample and ascorbic acid concentration (µg) were put along abscissa and ordinate respectively.

**DPPH assay**

Radical scavenging power of plant extract was determined by measuring absorbances of increasing concentrations (0 - 500 µg/ml) of extracts and ascorbic acid at 517 nm (Figure 2). The extract showed dose-dependent increase in scavenging activities with greater antioxidant capacity than ascorbic acid having IC\(_{50}\) values of 138.83 µg/ml and 100.49 µg/ml respectively (Table 1).

**HRSA assay**

Hydroxyl radical scavenging assay showed similar effect
Table 1. Inhibition concentration (IC$_{50}$) in DPPH and HRSA assays and effective concentration (EC$_{50}$) in TBA assay, FRAP assay and RPA assay, of ascorbic acid and plant extract.

| Assay     | Inhibition concentration (IC$_{50}$) in µg/ml | Ascorbic acid | Plant extract |
|-----------|---------------------------------------------|---------------|---------------|
| DPPH assay | 100.49                                      | 138.83        |
| HRSA assay | 131.42                                      | 353.82        |

**Effective concentration (EC$_{50}$) in µg/ml**

| Assay     | EC$_{50}$ in µg/ml |
|-----------|-------------------|
| TBA assay | 4371.0            |
| FRAP assay | 33.09            |
| RPA assay | 67.89             |

Table 2. Effect of methanol extract of *Fimbristylis miliacea* on castor oil induced diarrhoea in mice.

| Group     | Initial evacuation(min) (mean ± SD) | Evacuation classification | Evacuation index | % inhibition |
|-----------|-------------------------------------|---------------------------|------------------|-------------|
| Control   | 39.7±4.74                           | Solid: 3.50±0.76          | 23.5            | -           |
| Standard  | 177.5±6.0                           | Semi-solid: 2.67±0.96     | 9.83            | 58.16       |
| FM100     | 35.83±4.54                          | Liquid: 1.0±0.37          | 13.0            | 44.68       |
| FM200     | 27.67±11.18                         |                           | 9.50            | 59.57       |
| FM400     | 56.33±10.58                         |                           | 4.50            | 80.85       |

Here, 'FM' stands for methanol extract of *Fimbristylis miliacea* and data are presented as mean ± SEM. ANOVA was performed followed by Dunnett’s test and significant differences were represented by *p<0.05, **p<0.01, ***p<0.001 vs control group.

as DPPH assay exhibiting dose-dependent reduction of hydroxyl radicals. The extract was found to impart moderate effect capacity compared to standard ascorbic acid; IC$_{50}$ values were 353.82 and 131.42 µg/ml for extract and ascorbic acid respectively.

**TBA assay**

Thiobarbituric acid assay (TBA test) quantifies the amount of malondialdehyde, a product of lipid peroxidation, reacted with thiobarbituric acid. It is evident that plant extract demonstrated low antioxidant activity in comparison with ascorbic acid. EC$_{50}$ values were found 2159.50 µg/ml and 4371.00 µg/ml for plant extract and ascorbic acid respectively.

**FRAP assay**

FRAP assay demonstrated near equal magnitude of antioxidant capacity of both plant extract and ascorbic acid. EC$_{50}$ values were 31.59 and 33.09 µg/ml for extract and ascorbic acid respectively.

**RPA assay**

Dose dependent increase in reduction of ferric to ferrous was observed in both plant extract and ascorbic acid (Figure 2). The extract exhibited moderate antioxidant activity in this assay having EC$_{50}$ of 129.56 µg/ml, whereas ascorbic acid had 67.87 µg/ml.

**Antidiarrhoeal activity in mice model**

Table 2 shows the effect of methanol extract of *F. miliacea* on castor oil induced diarrhoea. Strong anti diarrhoeal effect was observed at doses of 200 and 400 mg/kg.bw. Standard drug loperamide and 200 mg/kg.bw extract inhibited diarrhea by nearly equal magnitude. Evacuation indices and percentage inhibition at 200 mg/kg.bw dose were 9.50 and 59.57% respectively while for loperamide, these values were 9.83 and 58.16% respectively. Increasing extract concentration to 400 mg/kg.bw showed a further increase in anti diarrhoeal effect; this highest extract dose demonstrated an EI of 4.50 and inhibited 80.85% diarrhea. Here, effect on severity of diarrhoea was determined by evacuation index (EI); the less the EI value, the stronger the anti diarrhoeal effect.

**DISCUSSION**

Phytoconstituents with different chemical compositions perform diverse functions in plant physiology. Besides
their beneficial roles for own system, these chemicals are also found to have promising pharmacological effects for human and other animals. Among various effects, protective role against oxidative stresses is of great importance. Reactive oxygen species in cells can initiate chain reactions damaging cellular components. Hydroxyl radical- a reactive oxygen species, is liable for significant cellular damage by lipid peroxidation. Antioxidant activities of phytoconstituents remove these reactive species and thus can cease the chain reactions. Plants from diverse genera are found to possess potent antioxidant activities (Bajalan et al., 2016; Barros et al., 2017; Fitriana et al., 2016; Jimenez-Gonzalez et al., 2018; Maisetta et al., 2019). Antioxidant activity of extract of F. miliacea was measured using five different assays as this activity is influenced by various factors such as test method and chemical composition of the extract; thus, multiple assays can reveal various mechanisms of antioxidant effects. In all antioxidant assays, extract of F. miliacea showed dose-dependent increase in antioxidant activity.

In DPPH and FRAP assay, the extract demonstrated comparable effect as ascorbic acid and in other three assays, moderate antioxidant effect was found. In DPPH assay, change of colour of the reaction mixture involves pairing of hydrogen from antioxidant and DPPH free radicals and high antioxidant effect is manifested by more DPPH reduction. FRAP assay depends on the conversion of ferric to ferrous ion. In HRSA assay, generation of hydroxyl free radicals is promoted in such a way that it is ultimately scavenged by plant extract or standard compound. Reducing power activity (RPA) assay is based on the reduction of ferric ion of potassium ferricyanide to ferrous ion. Thiobarbituric acid assay (TBA) measures the amount of malondialdehyde that is a secondary product of peroxide decomposition. Phytochemicals containing multiple hydroxyl groups such as phenolic, flavonoids, flavonols are very effective as antioxidant.

Hydrogen cation generated from hydroxyl groups of phenolics react with free radicals to neutralize them. Phenolic compounds from different plant extract also possess a wide range of pharmacological activities, for example, anticarcinogenic (Xie et al., 2020), antiatherosclerotic (Zhang et al., 2018), antibacterial (Rodriguez-Pérez et al., 2016), anti-inflammatory activities (Furtado et al., 2016), etc. Flavonoids are polyhydroxylated phenolic substances containing 2-phenyl-chromone nucleus and are widely found in plant. Flavonols, a subclass of flavonoids, are composed of the basic structure of 2-benzo-γ-pyrone. Presence of hydroxyl groups in flavonoids plays a protective role in plant cells against abiotic and biotic stresses. Total number of hydroxyl groups and other substitutions in the flavonoids significantly influence their antioxidant activity. Flavonoids interferes with activities of various enzymes such as microsomal monoxygenase, mitochondrial succinioxidase, NADH oxidase, etc (Aloud et al., 2018). These enzymes along with their normal activities generate reactive oxygen species; thus, inhibition of these enzymes reduces the production of free radicals. Antioxidant activity of plant extract is believed to elicit from high phenolic and flavonoid contents. High amount of total polyphenols in F. miliacea extract endorses moderate to strong antioxidant activity of its methanol extract.

Carotenoids- β-carotene and lycopene, comprise a family of pigmented compounds synthesized mainly in plants and also in small extent in microorganisms. Fruits and vegetables are the chief dietary sources of carotenoids. These compounds are found to have beneficial roles in prevention of many human diseases including cardiovascular diseases, cancer and other chronic diseases and a positive correlation has been reported between high dietary carotenoids content and low risk of chronic diseases (Gammone et al., 2017; Johnson, 2002; Rao and Rao, 2007): scavenging of reactive oxygen species by carotenoids are believed to the principle mechanism of their beneficial roles against various diseases. Additionally, these compounds can also regulate in cell growth regulation, modulate gene expression and elicit immune response, etc (Elliott, 2005).

In castor oil induced antidiarrheal experiment, castor oil causes increased fluid accumulation in intestine and aggravated peristaltic movement resulting in frequent defecation. Significant improvement in evacuation frequency and duration in castor oil induced diarrhea was demonstrated by methanol extract of F. miliacea and the effect is comparable to that of standard antidiarrheal drug loperamide. High content of tannin and flavonoids in our plant extract may be attributed for strong antidiarrheal effect. Tannins can form a coating on intestinal mucosa by reacting with local proteins, thus reducing gastric secretions (Sarin et al., 2013). Moreover, tannins and flavonoids reduce peristaltic motility (Kumar et al., 2010).

Conclusion

Methanol extract of F. miliacea contains significant amount of phenolics, flavonoids and tannins that impart antioxidant potentials of the species and strong anti diarrheal effect. The species also demonstrated antipyretic, antinociceptive and hypoglycemic activities. These findings certainly warrant comprehensive chromatographic analyses to identify potential bioactive compounds responsible for these pharmacological effects.

ABBREVIATIONS

DPPH, diphenyl picrylhydrazyl assay; HRSA, hydroxyl radical scavenging assay; RPA, reducing power assay; TBA, thiobarbituric acid assay; FRAP, ferric reducing antioxidant power assay; IC, inhibition concentration; EC,
CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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