

*Full Length Research Paper*

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

**Ummah Hafsa Mukta<sup>#</sup>, Roni Roy<sup>#</sup>, A. F. M. Shahid Ud Daula, Mahmuda Ferdous, Anwsha Chowdhury, Sohel Mia, Afroza Akter, Israt Jahan Liya and Mohammad Anwarul Basher\***

Department of Pharmacy, Noakhali Science and Technology University, Sonapur, Noakhali-3814, Bangladesh.

Received 24 December, 2019; Accepted 21 February, 2020

The present study determined phytochemical content, antioxidant and antidiarrhoeal 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,  $\beta$ -carotene, lycopene, chlorophyll- $\alpha$ , and chlorophyll- $\beta$  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  $\mu$ g/g, 0.66  $\mu$ g/g, 7.10 mg/g and 4.25 mg/g, respectively. Antioxidant potential was compared against ascorbic acid. IC<sub>50</sub> and EC<sub>50</sub> values were determined as indices of antioxidant potential using five experiments; IC<sub>50</sub> in 2,2-diphenyl-1-picrylhydrazyl (DPPH) and hydroxyl radical scavenging assay were 138.83 and 353.82  $\mu$ g/ml respectively. EC<sub>50</sub> of thiobarbituric acid assay, FRAP assay and reducing power assay were observed 2159.5, 31.59 and 129.56  $\mu$ g/ml respectively. Total antioxidant capacity was 155.35 mg/g AAE. Significant reduction ( $p \leq 0.001$ ) 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

\*Corresponding author. E-mail: m.anwar.basher@gmail.com. Tel: +8801768522866.

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 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, streams 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 antinociceptive effect (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 ( $\bullet\text{OH}$ ), peroxide ( $\text{ROO}\bullet$ ), superoxide ( $\text{O}_2\bullet^-$ )- 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, antidiarrheal, cytotoxic, hepatoprotective, antidiabetic effects. The present study was aimed to investigate antioxidant and antidiarrheal 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 (Ebrahimzadeh et al., 2008). A calibration curve was first formulated using different concentrations of gallic acid (0-300  $\mu\text{g}/\text{mL}$ ). 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 standard curve with different concentrations and thus both flavonoid and flavonol content are generally expressed as  $\mu\text{g}$  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 (Rezaeizadeh 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- $\alpha$ and chlorophyll- $\beta$**

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 (Lichtenthaler and Wellburn, 1983):

$$\text{Chlorophyll-}\alpha \text{ (mg/ml)} = 15.65A_{666} - 7.34A_{653}$$

$$\text{Chlorophyll-}\beta \text{ (mg/ml)} = 27.0A_{653} - 11.21A_{666}$$

#### **$\beta$ -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-

$$\beta\text{-carotene (mg/100 ml)} = 0.216A_{663} - 0.304A_{505} + 0.452A_{453}$$

$$\text{Lycopene (mg/100 ml)} = -0.0458A_{663} + 0.372A_{505} - 0.805A_{453}$$

Results were expressed as  $\mu\text{g}$  of  $\beta$ -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 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.

##### **Diphenyl picrylhydrazyl assay (DPPH)**

2,2-diphenyl-1-picryl-hydrazyl free radical scavenging assay, commonly known as DPPH assay, was performed by mixing 3 ml of DPPH working solution with 300  $\mu\text{l}$  of plant extract (1 mg/ml) followed by incubation for 30 min at room temperature and then absorbance was measured at 517 nm (Ahmed et al., 2015). Ascorbic acid was used as standard. Percentage of inhibition was calculated as:

$$\% \text{ of Inhibition} = (A_0 - A_1/A_0) \times 100$$

Here,  $A_0$  and  $A_1$  were the absorbances of control and sample respectively.

##### **Reducing power assay (RPA)**

Sample for RPA assay was prepared by mixing 1 ml extract with 2.5 ml of 200 mM phosphate buffer (pH 6.6) and 2.5 ml of 30 mM

potassium ferricyanide (Jayanthi and Lalitha, 2011). The mixture was incubated at 50°C for 20 min, 2.5 ml of trichloroacetic acid was then added to the mixture, and centrifuged at 3000 rpm for 10 min. Clear aliquot was pipetted out, and 2.5 ml of distilled water and 0.5 ml of 6 mM ferric chloride were added. Absorbance of this mixture was measured at 700 nm. Here, ascorbic acid was used as standard.

##### **Ferric reducing antioxidant power assay (FRAP)**

FRAP assay works by same mechanism as RPA assay and determines the ability of plant extract to reduce ferric tripyridyltriazine complexes to ferrous tripyridyltriazine (Benzie and Strain, 1999). In this assay, 300  $\mu\text{l}$  of extract was mixed with 3 ml of FRAP reagent (10 mM TPTZ, 40 mM HCl, 20 mM  $\text{FeCl}_3$ , pH 3.6). The mixture was then warmed at 37°C in oven. Upon cooling, absorbance was measured at 593 nm. Ascorbic acid was used as standard. Antioxidant capacity was expressed in  $\mu\text{g}$  ascorbic acid per g of sample.

##### **Hydroxyl radical scavenging assay (HRSA)**

1 ml of extract of different concentrations was mixed with 250  $\mu\text{l}$  of 6 mM  $\text{FeSO}_4$  and 0.5 ml of 6 mM  $\text{H}_2\text{O}_2$ . 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**

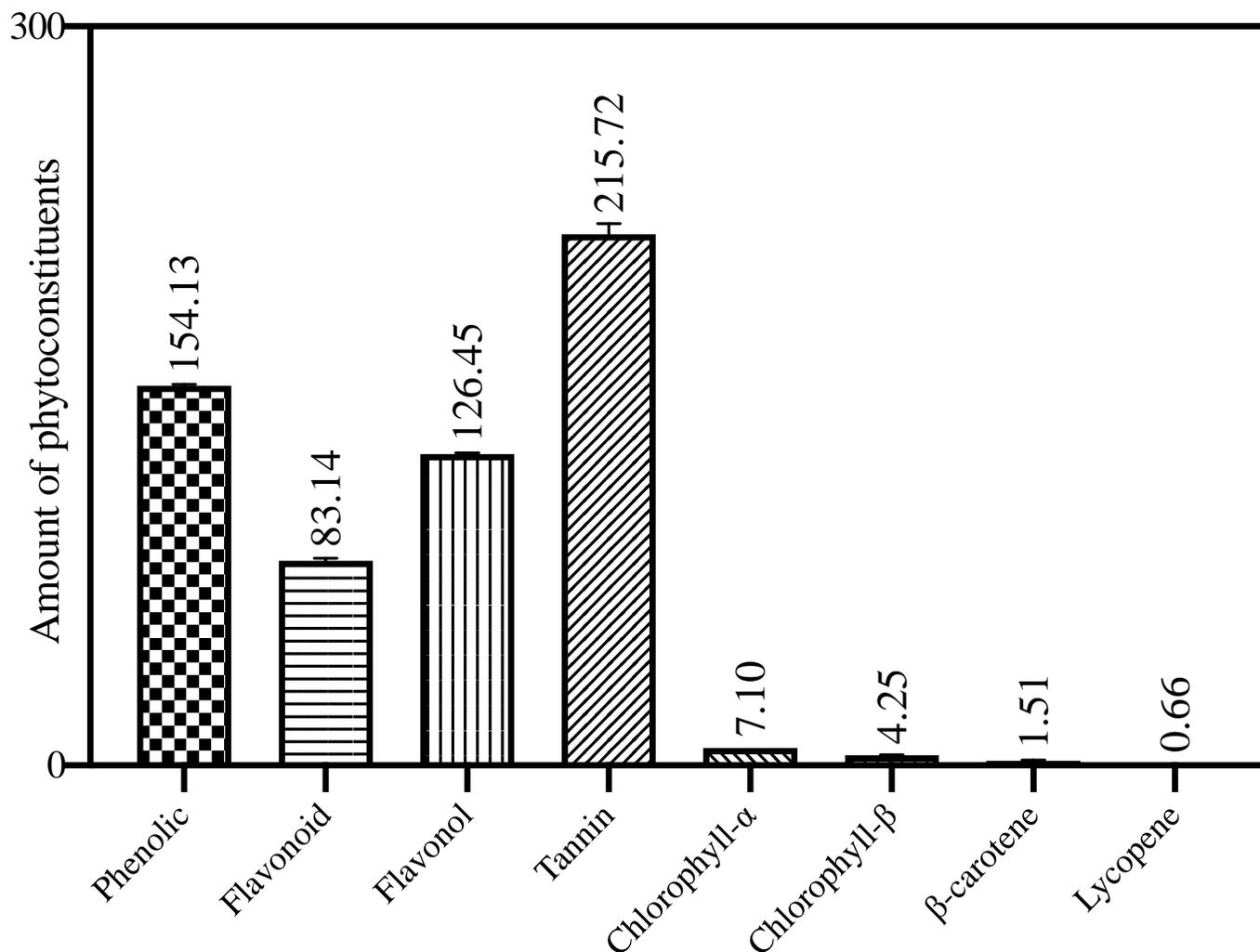
Reagent for TBA assay was prepared by adding 1 ml of 20% aqueous trichloroacetic acid with 2 ml of 0.67% aqueous thiobarbituric acid. 2 ml of plant extract was then mixed with this reagent (Mackeen et al., 2000). The mixture was subjected to boiling for 10 min. Upon cooling, the sample was centrifuged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

##### **Determination of $IC_{50}$ and $EC_{50}$ value**

$IC_{50}$  (inhibition concentration) value represents concentration of antioxidant necessary to reduce initial concentration by 50%. Low  $IC_{50}$  indicates high antioxidant activity.  $EC_{50}$  (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  $23 \pm 1^\circ\text{C}$  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.



**Figure 1.** Amount of phytoconstituents in methanol extract of *F. miliacea*. Here, phenolic and tannin were expressed as 'mg gallic acid equivalent'; flavonoid and flavonol were expressed as 'mg quercetin equivalent';  $\beta$ -carotene and lycopene in  $\mu\text{g}$  in 1 g of methanol extract; chlorophyll  $\alpha$  and chlorophyll  $\beta$  in mg in 1 g of methanol extract. Each sample was assayed in triplicate.

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:  $\text{EI} = 1 \times (\text{No 1. stool}) + 2 \times (\text{No 2. stool}) + 3 \times (\text{No 3. stool})$ . Percentage inhibition of diarrhea was calculated as  $(\text{EI of vehicle} - \text{EI of sample}) \times 100 / (\text{EI of vehicle})$  (Mbagwu and Adeyemi, 2008).

## 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.

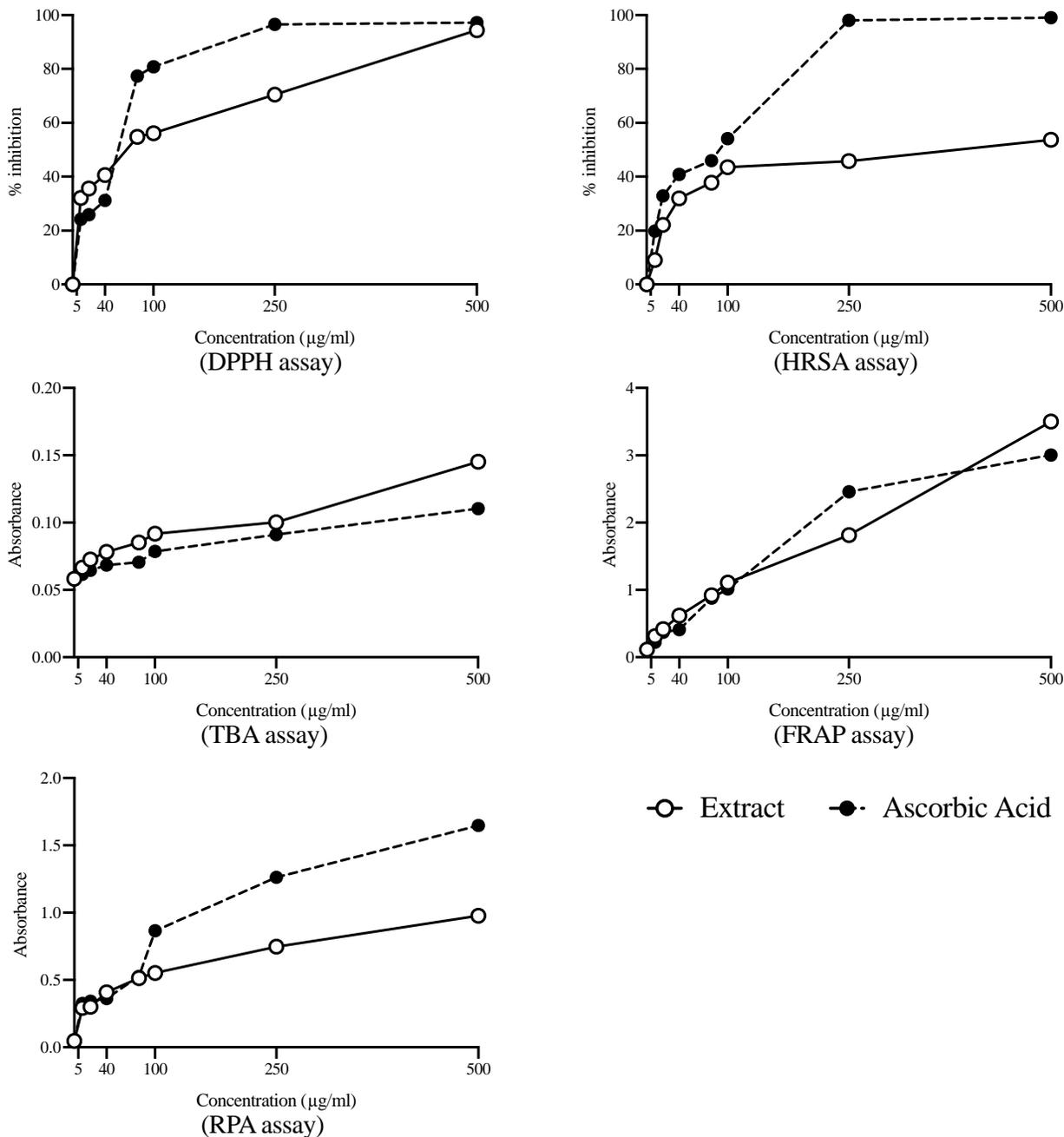
### Quantitative phytochemical contents

Figure 1 shows the amount of phenolic, flavonoid, flavonol, tannin, chlorophyll- $\alpha$ , chlorophyll- $\beta$ ,  $\beta$ -carotene and lycopene.

### Antioxidant activity of plant extract

#### Total antioxidant capacity

Total antioxidant capacity of the extract was found as  $155.35 \pm 0.006$  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 ( $\mu\text{g}$ ) 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  $\mu\text{g/ml}$ ) 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  $\text{IC}_{50}$  values of 138.83  $\mu\text{g/ml}$  and 100.49  $\mu\text{g/ml}$  respectively (Table 1).

**HRSA assay**

Hydroxyl radical scavenging assay showed similar effect

**Table 1.** Inhibition concentration (IC<sub>50</sub>) in DPPH and HRSA assays and effective concentration (EC<sub>50</sub>) in TBA assay, FRAP assay and RPA assay, of ascorbic acid and plant extract.

Assay	Inhibition concentration (IC <sub>50</sub> ) in µg/ml	
	Ascorbic acid	Plant extract
DPPH assay	100.49	138.83
HRSA assay	131.42	353.82
<b>Effective concentration (EC<sub>50</sub>) in µg/ml</b>		
TBA assay	4371.0	2159.5
FRAP assay	33.09	31.59
RPA assay	67.89	129.56

**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
		Solid	Semi-solid	Liquid		
Control	39.7±4.74	3.50±0.76	3.50±0.43	4.33±0.42	23.5	-
Standard	177.5±6.0	1.50±0.43	2.67±0.96	1.0±0.37	9.83	58.16
FM100	35.83±4.54	8.33±0.99 <sup>***</sup>	0.83±0.31 <sup>**</sup>	1.0±0.37 <sup>***</sup>	13.0	44.68
FM200	27.67±11.18	5.67±1.05	1.17±0.31 <sup>*</sup>	0.5±0.24 <sup>***</sup>	9.50	59.57
FM400	56.33±10.58	2.33±0.84	0.83±0.40 <sup>**</sup>	0.17±0.17 <sup>***</sup>	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 <sup>\*</sup>p<0.05, <sup>\*\*</sup>p<0.01, <sup>\*\*\*</sup>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<sub>50</sub> 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 compared with ascorbic acid. EC<sub>50</sub> 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<sub>50</sub> 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<sub>50</sub> 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 antidiarrheal 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 antidiarrheal 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 antidiarrheal 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 (Rodríguez-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- $\gamma$ -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 monooxygenase, mitochondrial succinoxidase, 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-  $\beta$ -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 antidiarrheal 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**,

effective concentration; **bw**, body weight; **min**, minute; **h**, hour; **g**, gram.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

## REFERENCES

- Ahmed D, Khan MM, Saeed R (2015). Comparative analysis of phenolics, flavonoids, and antioxidant and antibacterial potential of methanolic, hexanic and aqueous extracts from *Adiantum caudatum* leaves. *Antioxidants* 4(2):394-409. doi.org/10.3390/antiox4020394
- Al-Trad B, Al-Qudah MA, Al-Zoubi M, Muhaidat R, Qar J (2018). In-Vitro and In-Vivo Antioxidant Activity of the Butanolic Extract from the Stem of *Ephedra Althea*. *Biomedical and Pharmacology Journal* 11(3):1239-1245. dx.doi.org/10.13005/bpj/1485
- Aloud AA, Veeramani C, Govindasamy C, Alsaif MA, Al-Numair KS (2018). Galangin, a natural flavonoid reduces mitochondrial oxidative damage in streptozotocin-induced diabetic rats. *Redox Report* 23(1):29-34.
- Bajalan I, Mohammadi M, Alaei M, Pirbalouti AG (2016). Total phenolic and flavonoid contents and antioxidant activity of extracts from different populations of lavender. *Industrial Crops and Products* 87:255-260.
- Barros ImdC, Leite BH, Leite CF, Fagg CW, Gomes SM, Resck IS, Fonseca-Bazzo YM, Magalhães PO, Silveira D (2017). Chemical composition and antioxidant activity of extracts from *Erythroxylum suberosum* A. St. Hil. leaves. *Journal of Applied Pharmaceutical Science* 7(3):88-94.
- Benzie IF, Strain J (1999). [2] Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration, *Methods in enzymology*. Elsevier pp. 15-27. doi.org/10.1016/S0076-6879(99)99005-5
- Desjardins D, Cacho-Valadez B, Liu JL, Wang Y, Yee C, Bernard K, Khaki A, Breton L, Hekimi S (2017). Antioxidants reveal an inverted U-shaped dose-response relationship between reactive oxygen species levels and the rate of aging in *Caenorhabditis elegans*. *Aging Cell* 16(1):104-112. 10.1111/ace1.12528
- Ebrahimzadeh MA, Pourmorad F, Bekhradnia AR (2008). Iron chelating activity, phenol and flavonoid content of some medicinal plants from Iran. *African Journal of Biotechnology* 7(18):3188-3192.
- Elliott R (2005). Mechanisms of genomic and non-genomic actions of carotenoids. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease* 1740(2):147-154.
- Evans WC (2009). *Trease and Evan's Pharmacognosy*, 16th ed. Saunders Ltd., Nottingham, UK.
- Fitriana WD, Ersam T, Shimizu K, Fatmawati S (2016). Antioxidant activity of *Moringa oleifera* extracts. *Indonesian Journal of Chemistry* 16(3):297-301.
- Furtado AA, Torres-Rêgo M, Lima MC, Bitencourt MA, Estrela AB, da Silva NS, da Silva Siqueira EM, Tomaz JC, Lopes NP, Silva-Júnior AA (2016). Aqueous extract from *Ipomoea asarifolia* (Convolvulaceae) leaves and its phenolic compounds have anti-inflammatory activity in murine models of edema, peritonitis and air-pouch inflammation. *Journal of Ethnopharmacology* 192:225-235.
- Gammone MA, Pluchinotta FR, Bergante S, Tettamanti G, D'Orazio N (2017). Prevention of cardiovascular diseases with carotenoids. *Front. Bioscience (Schol. Ed.)* 9:165-171.
- Hung CH, Cheng SS, Cheung YT, Wuwongse S, Zhang NQ, Ho YS, Lee SM, Chang RC (2018). A reciprocal relationship between reactive oxygen species and mitochondrial dynamics in neurodegeneration. *Redox Biology* 14:7-19. 10.1016/j.redox.2017.08.010
- Jayanthi P, Lalitha P (2011). Reducing power of the solvent extracts of *Eichhornia crassipes* (Mart.) Solms. *International Journal of Pharmacy and Pharmaceutical Sciences* 3(3):126-128.
- Jimenez-Gonzalez FJ, Vélez-Gómez JM, Melchor-Moncada JJ, Veloza LA, Sepúlveda-Arias JC (2018). Antioxidant, anti-inflammatory, and antiproliferative activity of extracts obtained from *Tabebuia Rosea* (Bertol.) DC. *Pharmacognosy Magazine* 14(55):25.
- Johnson EJ (2002). The role of carotenoids in human health. *Nutrition in Clinical Care* 5(2):56-65. 10.1046/j.1523-5408.2002.00004.x
- Kumar B, Divakar K, Tiwari P, Salhan M, Goli D (2010). Evaluation of anti-diarrhoeal effect of aqueous and ethanolic extracts of fruit pulp of *Terminalia bellerica* in rats. *International Journal of Drug Development and Research* 2(4):769-779.
- Lichtenthaler HK, Wellburn AR (1983). Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochemical Society Transactions* 11(5):591-592. doi.org/10.1042/bst0110591
- Mackeen M, Ali A, Lajis N, Kawazu K, Hassan Z, Amran M, Habsah M, Mooi L, Mohamed S (2000). Antimicrobial, antioxidant, antitumour-promoting and cytotoxic activities of different plant part extracts of *Garcinia atroviridis* Griff. ex T. Anders. *Journal of Ethnopharmacology* 72(3):395-402. doi.org/10.1016/S0378-8741(00)00245-2
- Maisetta G, Batoni G, Caboni P, Esin S, Rinaldi AC, Zucca P (2019). Tannin profile, antioxidant properties, and antimicrobial activity of extracts from two Mediterranean species of parasitic plant *Cytinus*. *BMC Complementary and Alternative Medicine* 19(1):82.
- Mbagwu HO, Adeyemi OO (2008). Anti-diarrhoeal activity of the aqueous extract of *Mezoneuron benthamianum* Baill (Caesalpinaceae). *Journal of Ethnopharmacology* 116(1):16-20. 10.1016/j.jep.2007.10.037
- Mia S, Roy R, Akter A, Berek MA, Basher MA (2019). Hypoglycemic effect of leaf extract of *Fimbristylis miliacea* in mice model. *Clinical Phytoscience* 5 10.1186/s40816-019-0119-x
- Mohammed S, Manan FA (2015). Analysis of total phenolics, tannins and flavonoids from *Moringa oleifera* seed extract. *Journal of Chemical and Pharmaceutical Research* 7(1):135-7.
- Ni R, Cao T, Xiong S, Ma J, Fan GC, Lacefield JC, Lu Y, Le Tissier S, Peng T (2016). Therapeutic inhibition of mitochondrial reactive oxygen species with mito-TEMPO reduces diabetic cardiomyopathy. *Free Radical Biology Medicine* 90:12-23. 10.1016/j.freeradbiomed.2015.11.013
- Prasad S, Gupta SC, Tyagi AK (2017). Reactive oxygen species (ROS) and cancer: Role of antioxidative nutraceuticals. *Cancer Letters* 387:95-105. 10.1016/j.canlet.2016.03.042
- Rao AV, Rao LG (2007). Carotenoids and human health. *Pharmacological Research* 55(3):207-216.
- Rezaeizadeh A, Zuki A, Abdollahi M, Goh Y, Noordin M, Hamid M, Azmi T (2011). Determination of antioxidant activity in methanolic and chloroformic extracts of *Momordica charantia*. *African Journal of Biotechnology* 10(24):4932-4940.
- Rice-Evans CA, Miller NJ, Bolwell PG, Bramley PM, Pridham JB (1995). The relative antioxidant activities of plant-derived polyphenolic flavonoids. *Free Radical Research* 22(4):375-83. 10.3109/10715769509145649
- Rodríguez-Pérez C, Quirantes-Piné R, Uberos J, Jiménez-Sánchez C, Peña A, Segura-Carretero A (2016). Antibacterial activity of isolated phenolic compounds from cranberry (*Vaccinium macrocarpon*) against *Escherichia coli*. *Food and Function* 7(3):1564-1573.
- Roy R, Ud Daula A, Akter A, Sultana S, Berek MA, Liya IJ, Basher MA (2019). Antipyretic and anti-nociceptive effects of methanol extract of leaves of *Fimbristylis miliacea* in mice model. *Journal of Ethnopharmacology* 243:112080. 10.1016/j.jep.2019.112080
- Sarin RV, Narwal S, Bafna PA (2013). Anti-diarrhoeal activity of aqueous extract of *Ocimum kilimandscharicum*. *Journal of Ethnopharmacology* 148(1):223-228.
- Simpson DA, Inglis CA (2001). Cyperaceae of economic, ethnobotanical and horticultural importance: a checklist. *Kew Bulletin* 56(2):257-360. 10.2307/4110962
- Sofowora A, Ogunbodede E, Onayade A (2013). The role and place of medicinal plants in the strategies for disease prevention. *African Journal of Traditional, Complementary and Alternative Medicine* 10(5):210-229.
- Waterhouse DF (1994). Biological control of weeds: Southeast Asian prospects. The Australian Centre for International Agricultural Research, Canberra, Australia.

Xie Y, Jiang C, Chen X, Wu H, Bi S (2020). Preparation of Oligomeric Phenolic Compounds (DHPs) from Coniferin and Syringin and Characterization of their Anticancer Properties. *BioResources* 15(1):1791-1809.

Zhang TT, Zheng CY, Hu T, Jiang JG, Zhao JW, Zhu W (2018). Polyphenols from *Ilex latifolia* Thunb.(a Chinese bitter tea) exert anti-atherosclerotic activity through suppressing NF- $\kappa$ B activation and phosphorylation of ERK1/2 in macrophages. *RSC Medicinal Chemistry* 9(2):254-263.