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Evaluation of antioxidant, cytotoxic, thrombolytic and antimicrobial potentials of stem and root parts of *Phyllanthus fraternus* G.L. webster grown in Northern part of Bangladesh

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Abstract
Phyllanthus fraternus G.L. Webster belonging to the family of phyllanthaceae is commonly found in northern part of Bangladesh and has been extensive traditional use in folk medicine for the treatment of many diseased conditions. The aim of present study was to explore the quantitative phytochemical, antioxidant, cytotoxic, thrombolytic and antimicrobial properties as well as *in vitro* α-amylase inhibitory activity of methanolic extract of stem (MESP) and root (MERP)of *Phyllanthus fraternus*. MESP and MERP contained a good amount of phenolic, flavonoids, proanthocyanidins and flavonols compounds and showed as the strongest scavenging activity with an IC_{50} value of 23.26µg/ml and 8.22µg/ml for DPPH and of 12.698µg/ml and 10.67µg/ml for ABTS respectively. From *in vitro* clot lysis method MERP (33.17%) exhibited more potential result than MESP (11.76%) as compared to standard drug Streptokinase (68.92%). Furthermore, MESP and MERP inhibited α-amylase enzyme in a dose dependent manner and brine shrimp mortality rate was increased with increasing concentration of extracts. In addition, positive result of antibacterial activity against both Gram-positive and Gram-negative bacteria was observed with highest zone of inhibition at 150mg/10µl concentration. In summary, these findings suggest that MESP and MERP possess a wide range of pharmacologically important phytochemicals which exhibited strong antioxidant activity.

Keywords: *Phyllanthus fraternus*, MESP, MERP, phytoconstituents, cytotoxicity, thrombolytic potentials

Introduction
Since prehistoric times, all plants produce medicinal compounds that have continued to play a dominant role in the maintenance of human health. The World Health Organization estimates that about 80% of the world population use plant extracts or their active constituents as folk medicine in traditional therapies [1]. The study of medicinal plants including the proper selection of plants, preparation of crude extracts, phytochemical and biological screening have opened the door to the development of new drug for the health care products [2]. Due to present of bioactive secondary metabolites known as phytochemicals, the medicinal plants are used for the treatment of a number of human diseases such as diabetes, dysentery, diarrhea, asthma, bronchitis, inflammation, ulcers, malaria, prostate cancer, hypertension, atherosclerosis, infant brain ischemia [3]. Great in number of physiological and biochemical operations in the human body may responsible to produce centered-oxygen contained free radicals and other reactive oxygen species as byproducts [4] and these includes a number of chemically reactive molecules such as hydrogen peroxide (H_2O_2), superoxide (O_2^-) and hydroxyl radical (OH^-) etc. Excess free radicals attack biological molecules such as lipids, proteins and nucleic acids that lead to tissue or cellular injury [5]. Moreover, oxidative stress implicate with formation of hundred of disorders in humans including atherosclerosis, arthritis, central nervous system injury, gastritis, cancer, diabetes and AIDS [6]. In the last three decades even though many new antibiotics are produced by pharmacological industries but genetic ability of bacteria to acquire and transmit microbial resistance against therapeutic agents has increased a lot [7]. In this sense, scientific interests have been created on natural products due to their antioxidant, antimicrobial, cytotoxic and chemo preventive properties and hence there is a trend to substitute synthetic antioxidant with naturally occurring antioxidants due to their negative health effects. But scientific information on antioxidant properties of endemic plants is still

rather scarce and the potential of higher plants as a source for new drugs is still largely unexplored.

Phyllanthus fraternus is one of the important medicinal plants commonly known as Bhui -amlain Bangladesh, belongs to family Phyllanthaceae. It is small perennial herb with alternate compound leaves of small leaflets and axillary, fascicled, short pedicel led small flowers, grows wild as a weed throughout the country [8]. Different combinations of secondary metabolites are available to see in most of the herbs belonging to genus *Phyllanthus* which subject to their rendition of medicinal properties and isolated major class of bioactive compounds from these herbs are alkaloids, flavonoids, lignans, phenols, tannins and terpenes [9]. Different parts of *Phyllanthys fraternus* Webster such as leaf, stem and root have tremendous uses in traditional medication system of Bangladesh. But the folk medicinal practitioners use these plants without having knowledge about their side effects and toxicity. If it is possible to estimate the different types of secondary components and antioxidant activity with cytotoxic and antibacterial properties, we can properly use these plants for the treatment of various diseases such as cancer, heart disease, diabetic and alzeimers diseases and it will ultimately lead to the discovery of new drug. So in the present study, the methanolic extract of stem (MESP) and root (MERP) of *Phyllanthus fraternus* Webster have been selected for investigation and this research works have been designed to study the phytochemical profile as well an antioxidant, thrombolytic, cytotoxic, antidiabetic and antibacterial properties of MESP and MERP.

Materials and Methods

Chemicals and Reagents

Methanol; 2, 2-diphenyl-1-picrylhydrazyl (DPPH); 2, 2′-azino-bis (3-ethylbenzothiazoline-6 sulfonic acid) diammonium salt (ABTS•), AlCl3, Potassium per sulfate, Folin-Ciocalteus's phenol reagent, sodium carbonate were obtained from Sigma-Aldrich, Germany. Catechin and gallic acid were bought from Wako pure chemicals Ltd, Japan; Ascorbic acid from E-Merck, Germany; Streptokinase was purchased from Polamin Werk GmbH, Germany.

Collection and proper identification of the plant Sample

The methanolic extract of stem (MESP) and root (MERP) of *Phyllanthus fraternus* were selected for the present investigation. The plant samples were collected from the area of Rajshahi University campus in april-july, 2016. The plant was authenticated by Professor Dr. Gour Pada Ghosh, Department of Botany, Rajshahi University, Rajshahi.

Drying and pulverization of plant materials

The collected plant *Phyllanthus fraternus* was washed with water in such a way that adhering dirty materials were completely removed. After washing, plant samples were cut to get stem and root parts of plant and were sun dried for 7-10 days and finally kept in an electric oven for 72 hours at 40 ºC. Finally with the help of a grinding machine (FFC-15, China), the obtained dried materials were pulverized to prepare a coarse powder which were then stored in an airtight container for further use.

Extraction of powdered plant materials with methanol

The weight of total powdered from plant stem and root sample of *Phyllanthus fraternus* were 200 gm and 100 gm respectively. For cold extraction, each powdered material was kept immersed in 800 ml and 400 ml methanol respectively in an aspirator bottle at room temperature for 12 days with occasional shaking and stirring. Then the extract was filtered through a filter paper (Whatman No. 41) and concentrated with a rotary evaporator under reduced pressure at 50 ºC to obtain methanolic extract of stem (4.0 gm) and root (2.5 gm) of *Phyllanthus fraternus*.

Determination of total phenolic and flavonoid contents

The Folin–Ciocalteu modified method Singleton *et al.* [10] was applied to determine the total polyphenolic content (TPC) of MESP and MERP. Gallic acid (Sigma Aldrich, Germany) in the range of 1-32µg/ml concentrations was used for constructing the standard curve. The absorbance was measured at 760 nm. TPC was calculated as gallic acid equivalents ($y = 0.1236x + 0.0848$; $R^2=0.9965$) per gm of dried weight extracts.

Total flavonoids contents (TFLC) of MESP and MERP was determined by $AlCl_3$ colorimetric method as described by Dewnto *et al.* [11], using Catechin (Sigma Aldrich, Germany) as a standard reference in the range of 31.25-500µg/ml concentrations. The absorbance was read at 510 nm using a spectrophotometer. TFLC was expressed in terms of Catechin equivalent ($y=0.0051x+0.0859$, $R^2=0.9781$) per gm of dried weight extracts.

Determination of total proanthocyanidins and flavonol contents

Total proanthocyanidin contents (TPCC) of test samples were determined by colorimetric method [12]. Catechin, a reference standard (in the range of 18.73-300µg/ml) was used for calculating TPCC of the extracts as catechin equivalents ($y=0.0021x+0.0031$; $R^2=0.9965$) per gm of dried extracts. The absorbance was measured at 500 nm.

The total flavonol contents (TFC) of MESP and MERP was determined based on the method reported by Kumaran and Karunakaran *et al.* [13]. Quercetin was used as a standard in the range of 0.5-3.0µg/ml concentrations and TFC was expressed as quercetin equivalents ($y=0.2214x+0.005$; $R^2=0.9927$) per gm of dried extracts. The absorbance was measured at 440 nm.

Determination of antioxidant activity

DPPH and ABTS• free radical scavenging assay

Free radical scavenging activity of test samples was determined by DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging assay by established Choi *et al.* modified method [14]. 0.1 mM DPPH solution was prepared in methanol from where 3ml solution was mixed with 1 ml of extractives overwhelmed in methanol at different concentrations. Then reaction mixture was vortexed thoroughly and kept in the dark for 30 minutes at room temperature to complete the reactions. At the end, the absorbance was measured at 517nm by spectrophotometer. Here Catechin was used as a reference standard to compare.

The second test for determining the antioxidant capacity of samples was completed with ABTS•+ radical scavenging assay as described by Cai *et al.* [15]. Following the procedure, ABTS•+ solution was prepared by reacting 7mM ABTS stock solution with 2.45mM potassium persulfate. The obtained reaction mixture was left in the dark at room temperature for 12-16 hrs before use. The stabled ABTS•+ solution (for 2

days) was then diluted with water to get an absorbance of 0.7+0.02 at 734 nm. In the next step, 3ml of ABTS•+ solution was added to 1ml of test samples at various concentrations and mixed them vigorously. After standing for 6 minutes, the solution was ready to measure absorbance at 734 nm. Ascorbic acid was used as standard reference. All samples were experimented for triplicate in both assays.
Percentage of DPPH and ABTS●+ radical scavenging activity of the extracts was calculated using following formula:

Scavenging effect (%) = $[(A_{control} - A_{sample}) / A_{control})] \times 100$

Where $A_{control}$ is the absorbance of the control without sample and A_{sample} is the absorbance of the extractives/standard. Then percentage DPPH and ABTS scavenging activity were plotted against concentrations and IC_{50} was calculated from nonlinear regression curve which represents the effective concentration of extract causing 50% neutralization of radicals.

Thrombolytic activity
Experiments for clot lysis were carried as reported earlier [16] to investigate whether methanolic extract of MESP and MERP possess thrombolytic activity or not, compared with standard streptokinase. Briefly, 0.4gm extract from both samples were suspended individually in 10 ml of saline (0.98% saline). It was kept for overnight, and then filtered solution was collected as a stock. That time, 500 µl venous blood was collected from healthy human and transferred to previously weighted 20 eppendrof tubes. The tubes were then incubated for 2 hour at 37 °C. After that serum was removed carefully and again weight was taken of each tube with clot. (Clot weight = wt. of clot containing tube – wt. of tube alone). 100µl extract from each sample was added to each tube containing clot. For positive and negative control 100µl streptokinase and 100µl normal saline were added respectively. Again, all tubes were incubated for 90 min at 37 °C. After incubation, the released fluid was removed from above the tubes. Then each tubes weighed was taken to observe the difference in weight after clot disruption. Percentage of clot lysis was determined by following formula:

% Clot lysis = (Weight of the lysis clot /Weight of clot before lysis) × 100.

Brine shrimp lethality bioassay
Brine shrimp lethality bioassay (BSLA) was carried out for the investigation of general toxic property of the sample extracts according to published method [17]. Here *Artemia salina* (simple zoological organism) was used as a convenient monitor for screening the cytotoxicity. Following the method, 10 mg of the extract was prepared by dissolving them in 1 ml of distilled water to get a concentration of 10 µg/ µl and solution of different concentrations such as 10, 25, 50 and 100 and 200 µg/ml were obtained in different experimental vials and in each vial, 15 matured brine shrimp naupolii (*Artemia salina*) were poured. After 24h.of inoculation, a magnifying glass was used to inspect each vial containing nauplii and the number of survived nauplii was counted. Depending on the obtained data, the percentage of mortality of nauplii was calculated for each concentration and the LD_{50} (Median lethal dose) values were determined using probit analysis [18]. In this case, gallic acid was used as a standard reference.

Alpha amylase inhibitory test: Starch-Iodine Color Assay
To explore the antidiabetic potentials of selected plants, *in vitro* starch iodine color test was carried out to search α-amylase inhibitory property based on the procedure of Xiao *et al.* [19]. According to the procedure, 300µl plant extracts at various concentrations were added to 200µl α-amylase solution (dissolved in 0.02M sodium phosphate buffer, pH=6.9) and 500µl of soluble starch (1% (w/v). The reaction mixture were incubated at 37 °C for 1 hour. Then 500 µl of 3M HCl and 500 µL of iodine reagent (5mM I_2 and 5mM KI) were added to stop the enzymatic reaction. Absorbance was measured at 620 nm followed by the color change of the mixture. Glitazid was used as a positive control at a concentration range of 31.25-500 µg/ml. The results were expressed as % inhibition that calculated using the following formula:

% Inhibition of α-amylase activity = $[(A_S - A_{B1})/(A_{B2} - A_{B1})] \times 100$

Where A_S is the absorbance of test samples, A_{B1} is the absorbance of blank with α-Amylase solution and AB2 is the absorbance of blank without α-amylase solution.

Antibacterial assay
A simple and well-standardized disk diffusion susceptibility method was used for the determination of the antimicrobial activity of test samples and antibiotics [20]. Several pathogenic bacteria including Gram-positive (*Staphylococcus aureus, Bacillus subtilis, Staphylococcus pyogenes)* and Gram-negative (*Escherichia coli, Agrobacterium species, Shigella dysenteriae, Salmonella typhi*) were selected to examine antibacterial activity of root and stem parts. In our investigation, Azithromycin (standard) disc (15µg/discs) was used as a positive control whereas blank filter paper saturated with methanol was used as negative control to ensure that the filter paper itself was not active. Sample discs were saturated with crude methanol extracts at the concentrations of 50 mg/10µl, 100mg/10µl and 150 mg/10µl, respectively for each extract. Gently the crude extract discs and standard discs were placed on the solidified agar plates cultured with selected organisms. Finally, the plates were incubated at 37.5 ºC for 24 hours so that maximum growth of the organism occurred. The test materials were found to show inhibitory effects against the growth of the microorganisms and a clear, distinct zone of inhibitions visualized surrounding the medium that was used to measure the antimicrobial effects of the extracts. The antibacterial activities were determined by measuring the diameter of inhibitory zones in term of mm with a transparent scale.

Statistical Analysis
Values were expressed as mean of samples analyzed ± SD (Standard deviation).SPSS statistical software (15 version)was performed to carry outstatistical analysis with one way analysis of variance (Anova) followed by Dunnett's't' test. $P<0.05$ were considered to be statistically significant.

Results
Total phenolic, flavonoid, proanthocyanidins and flavonol contents
MESP and MERP were standardized for their contents of phenolic, flavonoid, proanthocyanidins and flavonol compounds. In the present study, figure 1 showed that MERP contained the higher content of phenolics(18.80±0.36 mg of

GAE/g of dry extract) than MESP (10.32±0.64 mg of GAE/g of dry extract) extracts. Also content of proanthocyanidins was higher in MERP (152.017±2.91mg of Catechin /g of dry extract) than MESP (40.90±0.83mg of Catechin /g of dry extract).On the contrary, MESP (164.73±1.18mg of Catechin /g of dry extract) contained higher amount of flavonoids compounds than MERP (55.44±1.57mg of Catechin /g of dry extract). In addition, MESP have higher content of flavonols (15.39±0.12mg of Quercetine /g of dry extract) compounds than MERP (4.55±.080mg of Quercetine /g of dry extract).

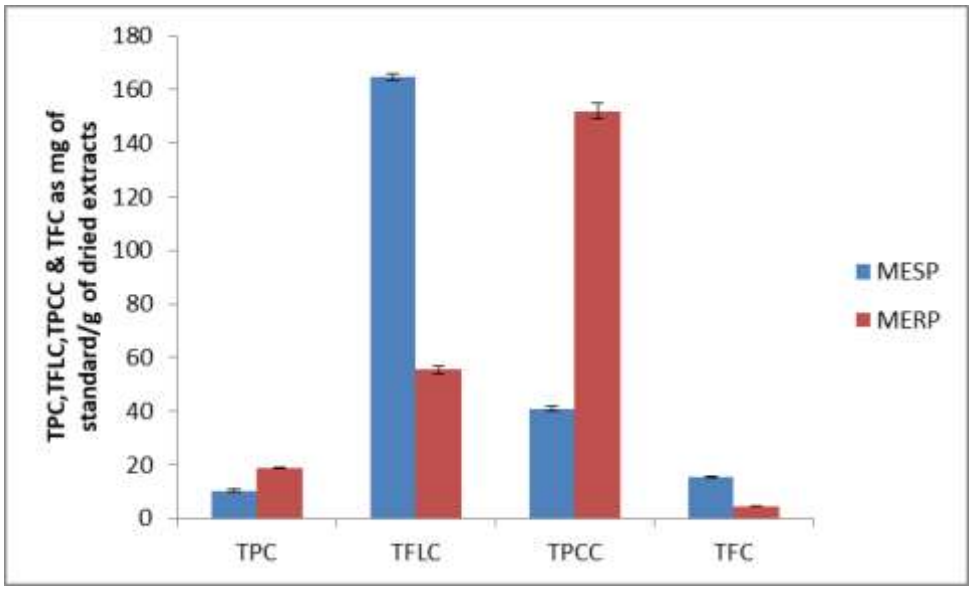
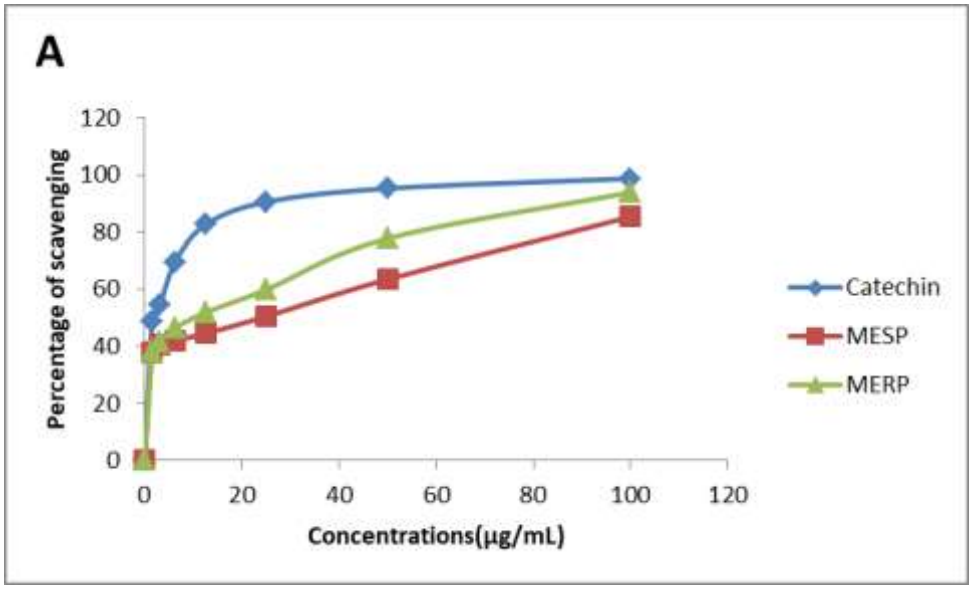


Fig 1: Total phenolic, flavonoid, proanthocyanidins and flavonol contents in MESP and MERP

Antioxidant properties

Radical scavenging activities are very important to prevent the deleterious role of free radical in different diseases including cancer. DPPH• free radical scavenging is an accepted mechanism by which antioxidants act to inhibit lipid peroxidation. Figure2(A) showed the free radical scavenging activity of MESP, MERP and Catechin. Plant samples and standard exhibited antioxidant activities in a dose dependent manner and positive result strongly support that samples were free radical scavengers. The scavenging activity of MESP and MERP was moderate when compared with standard Catechin. The IC_{50} values of MESP, MERP and Catechin were 23.26µg/ml, 8.22µg/ml and 2.65µg/ml, respectively.

ABTS• is more reactive than DPPH and unlike DPPH reaction, the reaction with ABTS• involve an electron transfer process [21]. The ABTS radical scavenging activity of MESP, MERP and Ascorbic acid as standard is represented in figure 2(B). The scavenging activity increased with increasing the concentrations of test materials. In lower concentration, MESP and MERP exhibited the lower scavenging properties when compared with vitamin C but in higher concentration showed greater scavenging properties than the standard. The IC_{50}values of MESP, MERP and Ascorbic acid were 12.698µg/ml, 10.67µg/ml and 4.658µg/ml respectively.



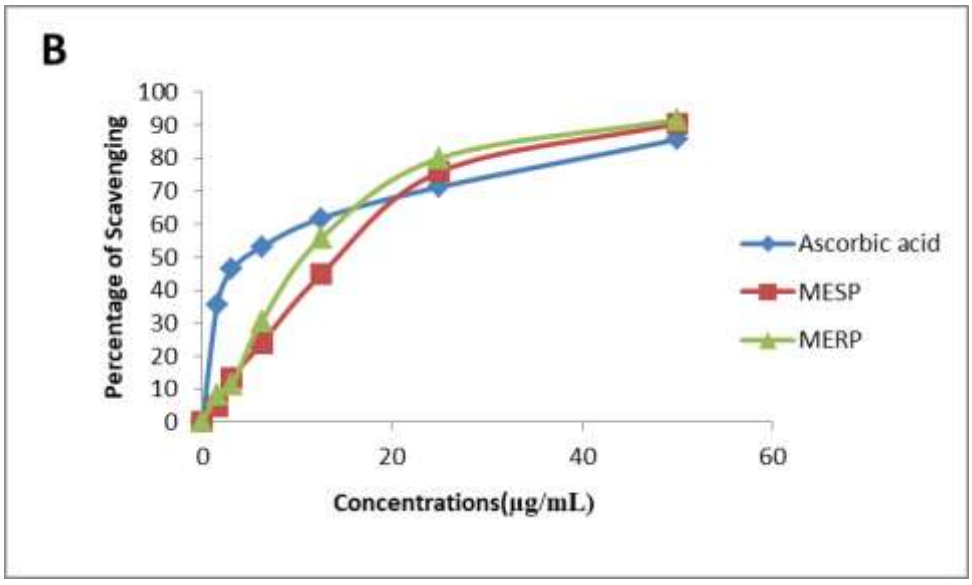


Fig 2: Determinations of (A) DPPH radical scavenging activity and (B) ABTS+ radical scavenging activity of MESP, MERP and Standard. Data are expressed as mean ± SD (n = 3) for all experiments. IC_{50} values were calculated from this nonlinear regression curve

Thrombolytic activity test

The percentage of weight loss of clot after application of extract solutions was taken as the functional indication of thrombolytic activity. Percent of clot lysis obtained after treating clots with MESP and MERP was shown in figure 3. From clot lysis experiment it was evident that MESP and MERP showed significant thrombolytic activity compared to the negative control. Streptokinase as a positive control showed 68.92% and normal saline as a negative control exhibited negligible 6.79% clot lysis activity. Treatment with MESP and MERP showed 11.76% and 33.17% clot lysis respectively which were in significant at $P<0.05$ compared with negative control.

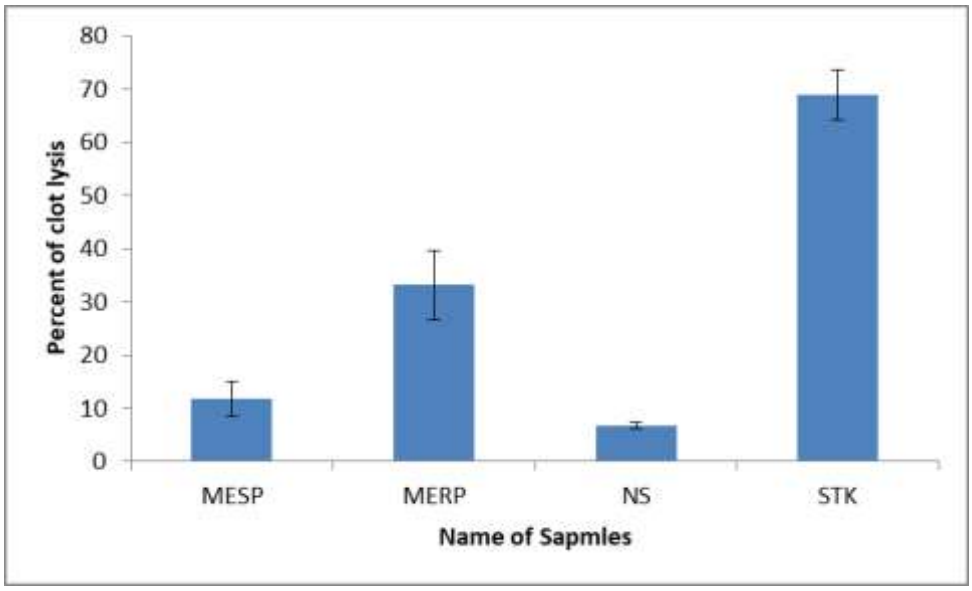


Fig 3: Clot lysis of blood samples of normal subjects by MESP, MERP, streptokinase (STK) and normal saline.

Alpha-amylase inhibition

Alpha-amylase inhibitory activity of test samples and Glitazid (as a standard) are shown in table 1.One of the most important therapeutic approaches to decrease postprandial hyperglycemia is the retardation of glucose absorption through inhibition of carbohydrate-hydrolyzing enzymes either α-amylase or α-glucosidase [22]. Our investigation in starch iodine color assay provides the evidence of α-amylase inhibitory activities of *P. Fraternus* extracts in a dose dependent manner and exhibited a concentration-dependent increase in percent inhibition of α-amylase activity with an IC_{50} values of 372.81 µg/ml, 110.69 µg/ml and 130.48µg/ml for MESP, MERP and standard respectively (Table 1). MERP have quite better effect on α-amylase than MESP.

Table 1: Alpha amylase inhibitory activity of MESP, MERP and Glitazid

Names of Sample	Concentration (µg/ml)	Percentage of inhibition (%)	IC_{50}(µg/ml)
MESP	50	12.11±1.22	372.81
	100	23.26±0.44	
	200	40.66±0.54	
	400	54.35±0.54	

	600	73.20±2.11	
	800	84.17±3.01	
MERP	50	32.05±2.31	110.69
	100	55.34±0.43	
	200	64.56±0.91	
	400	81.28±0.46	
	600	87.3±1.48	
	800	93.34±1.88	
Standard (Glitazid)	31.25	11.76±3.23	130.48
	62.5	27.14±4.14	
	125	49.60±0.61	
	250	64.99±0.88	
	500	84.67±0.95	

Data are expressed as mean ± SD (Standard Deviation)

Brine shrimp lethality bioassay

Following the procedure of Kabir *et al.* the lethality of crude methanol extract of *P. fraternus* to brine shrimp was determined on *A. salina* after 24hrs of exposure the samples and positive control, Gallic acid. The results of *P. fraternus* two extracts (concentrations of 10, 25, 50, 100 and 200µg/ml respectively) are shown in table 2. The percentage of mortality increased with an increased in concentration. The crude MESP and MERP showed better cytotoxic activity with LD_{50} values of 93.24µg/ml and 41.08µg/ml respectively in comparison with Gallic acid whose LD_{50} value is 4.53µg/ml (Table 3).

Table 2: Probit mortality data of MESP and MERP on brine shrimp nauplii

Dose µg/ml	Log dose	No of nauplii taken	No. of nauplii killed		% of mortality	
			MESP	MERP	MESP	MERP
10	0.9999	15	4	5	26.67	33.33
25	1.3010	15	5	8	33.33	53.33
50	1.6020	15	7	9	46.67	60
100	1.7781	15	10	11	66.67	73.33
200	1.9030	15	11	14	73.3	93.33

Table 3: Concentration dependent cytotoxic potential of crude MESP, MERP and Gallic acid against Brine shrimps nauplii by Probit

Test sample	LD_{50} (µg/ml)	95% Confidence limits (µg/ml) Lower-Upper	Regression equation	χ^2 value (Degrees of freedom)
Gallic acid	4.53	3.330 - 6.15	y=3.93 + 1.62x	0.049 (1)
MESP	93.24	54.75 - 158.80	y= 2.025+1.510x	0.369(3)
MERP	41.08	26.15 - 64.54	y= 2.413+1.602x	1.095(3)

Antimicrobial activity

In vitro antibacterial study of MESP and MERP showed the mild to moderate broad spectrum antibacterial activity against both Gram-positive and Gram-negative bacteria in comparison to standard antibiotic disc Azithromycin (K-15 µg/disc). The results obtained are shown in table 4. The produced zone of inhibitions of MESP were observed in the range of 10-21mm,7-16mm and 5-13mm for concentrations of 150mg/disc, 100 mg/disc and 50mg/disc respectively. The zone of inhibition of MERP were received in the range of 9-17mm,6-11mm and 4-11mm for concentrations of 150mg/disc, 100 mg/disc and 50mg/disc respectively. Both of plant materials showed less but significant inhibition against mentioned bacteria than Azithromycin (23-30mm for 15µg/disc concentration). Moreover, MESP is more potent against pathogenic bacteria than MERP (Table 4).

Table 4: Antibacterial activity of MESP and MERP

Name of Bacteria	Zone of inhibitions(mm)						
	MESP			MERP			Azithromycin
	50mg/disc	100mg/disc	150mg/disc	50mg/disc	100mg/disc	150mg/disc	15µg/disc
Staphylococcus aureus	13±1.22	15±0.54	18±0.72	8±0.56	10±0.63	13±4.23	27±1.34
Bacillus subtilis	10±0.97	16±2.40	19±2.81	11±0.64	11±4.11	15±0.66	23±2.88
Staphylococcus pyogenes	5±2.02	7±1.54	10±0.91	7±1.22	8±3.43	11±0.49	25±4.01
Escherichia coli	8±0.97	15±0.97	21±0.82	4±1.01	9±0.66	12±0.91	28±3.00
Agrobacterium species	9±1.26	12±0.69	17±0.55	7±0.23	11±0.52	12±3.77	28±0.78
Shigella dysenteriae	11±0.88	14±1.43	16±1.02	5±0.78	6±1.75	9±2.53	30±1.34
Salmonella typhi	8±2.32	12±0.40	13±0.37	9±3.16	10±3.05	17±0.44	27±0.55

Data are presented as the mean ± SD (standard Deviation) of three independent experiments.

Discussion

Phenolic compounds are important plant derived secondary metabolites that have efficient role in preventing chronic illnesses such as cardiovascular disease, certain type of cancers, neurodegenerative disease, and diabetes and their derivatives likely to show significant role in defense against pathogens and cell wall integrity [23]. Another popular phytochemical known as flavonoids have been shown to possess anti-oxidant, anti-inflammatory, anti-cancer and anti-microbial effects and display other pharmacological effects that plant possesses [24]. The efficient antioxidant activities of flavonoids are capable to prevent or trap superoxide anion

(O_2), hydroxyl (OH), peroxyl (ROO) and alcohoxyl (RO) radicals [23]. In addition, dietary proanthocyanidins and flavonols offer significant cardiovascular health benefits [25]. The present study revealed that MESP and MERP contain significant to rich amount of phenolic, flavonoids, proanthocyanidins and flavonol compounds which fascinating its free radical scavenging capability. The results of current study are in good contract with previous studies on other plant materials [26].

In DPPH scavenging assay, antioxidants present in the extract are thought to act as hydrogen donor and responsible for the reduction of DPPH. DPPH scavenging ability may depend on the amount of flavonoids and total phenolic content in these extracts. The results are in agreement with other reports [27] in which extracts enriched with flavonoids and phenolics showed DPPH$^{\bullet}$ scavenging properties. The results obtained from the present investigation revealed that MESP and MERP are free radical scavengers and able to react with the DPPH radical.

The ABTS scavenging assay is commonly used to measure the radical scavenging activity of hydrogen donating and chain breaking antioxidants in plant extract [28]. The present study demonstrated the potential ABTS free radical scavenging activity of MESP and MERP that increase with increasing the concentration of sample extract (Figure 2B). So MESP and MERP are considered as significant source of hydrogen donating and chain breaking antioxidant and other phytoconstituent. Compounds of natural origin containing antioxidant properties have been reported to be the important therapeutic intervention for cancer [29]. So MESP and MERP can be considered as important natural sources of cancer treatment instead of synthetic compounds because of their diverse side effects. Different agroclimatic conditions from different geographical positions can result in diverse phytochemical content and as it is well established that phytochemical content attributes to the antioxidant activities therefore, resulting difference in phytoconstituents might cause ultimate variations in antioxidant potentialities [30]. Considering the variations in phytochemical contents of *Phyllanthus fraternus* G.L. Webster stems and roots from different region around the world, it is overt that antioxidant properties must also vary with different geographical positions.

In vitro thrombolytic study on MESP and MERP demonestrated that the extracts showed moderate to significant clot lysis effect when compared with standard drug streptokinase. The thrombolytic activity exhibited by samples may be related to the presence of flavonoid constituents because the flavonoids can help to reduce atherosclerosis leading to heart attack or stroke [31]. So, methanol extracts of *P. fraternus* can be found to useful for treatment of cardiovascular disease.

In starch iodine color assay, MESP and MERP showed α-amylase inhibitory activity and the percentage of inhibition against α-amylase increased with increasing the concentration (Table 1). However, important chemical phytoconstituent such as polyphenols, flavonoids and glycosides may be responsible for their enzyme inhibitory activity. The plant based *α*-amylase inhibitor offers a prospective therapeutic approach for the management of diabetics [32]. So, the present study supports the concepts that stem and roots methanol extracts of *P. fraternus* could be useful in diabetic treatment and might be a good alternative to avoid adverse side effects of synthetic compound.

Brine shrimp lethality bioassay test of MESP and MERP exhibited promising cytotoxic lethal activity against naupolli. The significant lethal effects of sample materials (LD_{50} values less than 100 µg/ml) (Table 3) to brine shrimp indicates that crude extracts contain potent bioactive and probably insecticidal compounds [33]. BSLA results may guide the researchers which plants extracts/fractions will get priority for further fractionation and isolation of these bioactive compounds. Moreover, a number of novel antitumor and pesticide natural products have now been isolated using this bioassay [34]. This BSLA results revealed that MESP and MERP have significant cytotoxic effect and have also merit for further investigation on the isolation of active principle.

Plants exhibit significant medicinal properties due to their various complex chemical substances exclusively accumulated in different parts of them and on human body, they can produce marked healing action [35]. The inner most plant originated complex chemical substances are antibacterial compounds that have enormous therapeutical potential to defense against pathogens without side effects that are often associated with synthetic antimicrobials [36]. Phytochemical studies revealed that MESP and MERP contained significant phenolics and flavonoids content which can be attributed to their mild to moderate broad spectrum antibacterial activity because phenolics and flavonoids can complex with extracellular and soluble proteins as well as with bacterial cell wall [37]. In the present work the methanol stem and root extracts of *P. fraternus* were used for antimicrobial activity against seven bacterial strains. From the above discussion, we can say that MESP and MERP can be used to source antibiotic substances for drug development and could be a source for the industrial manufacture of drugs useful in the chemotherapy of some microbial infection.

Conclusion

From the results of present investigation, it can be concluded that antioxidant and α-amylase inhibitory activity of methanol extract of stem and root of *Phyllanthus fraternus* Webster render its suitability to be considered as a source for the development of anticancer and antidiabetic drug. Also antimicrobial activity against all test microorganisms is an indication that the extracts can be a source of very potent antibiotic substances that can be used against drug resistant microorganisms. However, it is necessary to carry out immense research on stem and root to isolate the active compounds responsible for antioxidant, antidiabetic and antimicrobial potentials. In future, our research will be forwarded along this direction.

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