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Phenolic Compound, Free radical assay, Anti-microbial and Anti-fungal Investigation of *Pterospermum semisagittatum*: A Herbal Flora of Bangladesh

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ABSTRACT
The present study was on *Pterospermum semisagittatum* investigated for its phenolic content, free radical scavenging (DPPH) assay, Antimicrobial and fungal activity. The highest phenolic content was found in the pet ether (PESF) (4.25±0.08 mg) followed by carbon tetrachloride soluble partitionate (CTCSF) (2.70±0.27 mg), chloroform soluble partitionate (CSF) (0.56±0.29 mg), methanolic extract (ME) (2.41±0.14 mg) and aqueous soluble partitionate (AQSF) (1.67±0.06 mg). In free radical scavenging (DPPH) assay, methanolic extract (ME), carbon tetrachloride soluble partitionate (CTCSF), pet ether (PESF), chloroform soluble partitionate (CSF) and aqueous soluble partitionate AQSF respectively, IC_{50} was 121.50±0.71, 112.50±1.14, 102.50±0.65 and 53.50±2.12. In Anti-microbial and anti-fungal investigation only chloroform soluble partitionate observed the Slight amount of activity. This study profound that *Pterospermum semisagittatum* has the remarkable presence of phenolic and antioxidant compound on preliminary investigational evaluation.

Keywords: *Pterospermum semisagittatum*, Antioxidant, DPPH, Phenolic Content, Anti-microbial activity, Anti-fungal activity.

1. Introduction
Medicinal plants have bestowed a good deal of arising new medicinal molecules. Scientist look around plants, herbs, microorganisms, marine resources etc. to incorporate new lead compounds. Bangladesh is blessed with numerous medicinal plants used for traditional healing, but very few are light of research [1]. In plants antioxidant activity plays a significant role in biological molecules brings about to, hydroxyl radicals, superoxide, nitric acid and various reactive oxygen varieties (ROS) i.e. hypochloric acid, hydrogen peroxide, and proxynitrite[2,3]. Also Phenolic intensifies are widely distributed in the plant kingdom. Plant secondary lipid precursors and aromatic amino acids. Phenolic compounds build an important portion of the secondary plant compounds. Lignin, a complex polymer of phenylpropane units is, quantitatively, the most important phenolic compound in plants. Other abundant compound classes are the flavonoids, coumarins, stilbenes and polyflavonoids. Now a day scientist has investigate medicinal plants for new antimicrobial activities [4-5].
The flora for example *Pterospermum semisagittatum* is endogenous in Bangladesh. This plant widely used in Herbal medicine as for gastrointestinal disorders, respiratory tract disorders, skin disorders, burning sensations, hepatic disorders, malaria, rheumatic pain cancer, tumor and heart palpitation etc. [6]. In these Study, the antioxidant, poly phenol and antimicrobial activity of *Pterospermum semisagittatum* was examined.

2. Materials and Methods
2.1 Plant collection
The leaves of *Pterospermum semisagittatum* (family Sterculiaceae) were accumulated from Dhaka, Bangladesh area. A voucher specimen (DACB-39205) has been banked in the National Herbarium, Dhaka.

2.2 Reagents and chemicals
All chemicals, i.e. methanol, n-hexane, carbon tetrachloride, chloroform, Gallic acid, 1, 1- diphenyl-2- picrylhydrazyl (DPPH), Folin-Ciocalteu reagent, butylated hydroxytoluene (BHT), ascorbic acid and other reagents used in these experiments were of the highest analytical grade.

2.3 plant Extraction and Isolation
The sun dried powdered leaves approximately 500 gm were macerated in 1.5 L of methanol for a week. The extract was filtered a through fresh cotton bed and finally with Whatman filter paper number 1 and concentrated with a rotary evaporator at reduced temperature and pressure. An aliquot (5 gm) of the concentrated methanol extract was fractionated [7]. Partition protocol and the resultant were evaporated to dryness with a rotary evaporator to yield to which petroleum ether (650.0 mg), carbon tetrachloride (950.0 mg), chloroform (450.0 mg) and aqueous (2.05 gm) soluble material from the crude methanol extract of leaves.

2.4 Phenolic Content Investigation
Total phenolic content of *Pterospermum semisagittatum* leaf extracts was assessed by using the method Demiary *et al*. (2009) and Skerget et al. (2005)[8,9]. Necessitating gallic acid as a standard and Folin-Ciocalteu reagent as an oxidizing agent. To 0.5 ml of extract solution (2 mg/ml) in water, 2.5 ml of Folin-Ciocalteu reagent (10 times diluted with water) and 2.0 ml of sodium carbonate (7.5 % w/v) solution were added. After 20 minutes of incubation at 25^0C and the absorbance was assessed using a UV visible spectrophotometer at 760 nm. Total phenolics were quantified by calibration curve obtained from the known concentrations of gallic acid (0-100 µg/ml) and were expressed as gm of GAE (gallic acid equivalent) / 100 gm of the dehydrated extract.

2.5 Free radical scavenging (DPPH) Investigation
The free radical scavenging activity of the extract, based on the scavenging activity of the stable 1, 1- diphenyl-2- picrylhydrazyl (DPPH) free radical, was determined by the method described by Braca *et al*. (2001) [10]. 2.0 ml of a methanol solution of the extract at different concentration (500 to 0.977 µg ml) were mixed with 3.0 ml of a DPPH methanol solution (20µg/ml). After 30 minutes reaction period at room temperature in a dark place the absorbance was measured against at 517 nm against methanol as blank by UV spectrophotometer. The percentage inhibition activity was calculated from $[(A_0–A_1)/A_0] \times 100$, where A_0 is the absorbance of the control, and A_1 is the absorbance of the extract/ standard. Then the inhibition curves were prepared and IC_{50} values were calculated. BHT was used as positive control.

2.6 The antimicrobial and fungal Investigation
The bacterial and fungal strains used for the experiment were collected as pure cultures from the Institute of Nutrition and Food Science (INFS), University of Dhaka. Standard disc of Ciprofloxacin (30 µg/disc) and blank discs impregnated with solvents followed by evaporation were used as positive and negative control. Both gram positive, gram-negative and fungal activity were taken for the test and they are listed in the table 2 the test material having antimicrobial activity and fungal activity inhibited the growth of the microorganisms and a clear, distinct zone of inhibition was visualized surrounding the discs. The antimicrobial activity of the test agents was determined by measuring the diameter of the zone of inhibition expressed in mm. All method as following Ayafor *et al*. (1972) and Bauer et al. (1966) [11, 12].

3. Result and Discussion
The methanol extract of leaf of *Pterospermum semisagittatum* and its different partitionates i.e. aqueous (AQSF), chloroform (CSF), carbon tetrachloride (CTCSF), and soluble partitionates pet ether (PESF) were subjected to total phenolic activity, Free radical Scavenging activity and Anti-microbial activity. Based on the absorbance values of the various extract solutions, reacted with Folin-Ciocalteu reagent and Calculated with the standard solutions of gallic acid curve (R^2=0.9985).The colorimetric analysis of the total phenolic contents are acceded Figure 1 and As well table 1.Among all extractives of *Pterospermum semisagittatum*, the highest phenolic content was found in PESF (4.25±0.08 mg of GAE / gm of dried extracts) followed by CTCSF (2.70±0.27 mg of GAE / gm of dried extracts). A significant amount of phenolic compounds were present in CSF (0.56±0.29 mg of GAE / gm of dried extracts), ME (2.41±0.14 mg of GAE / gm of dried extracts) and AQSF (1.67±0.06 mg of GAE / gm of dried extracts).
In free radical scavenging (DPPH) assay, methanolic extract (ME), Carbon tetrachloride soluble partitionate (CTCSF), pet ether (PESF), chloroform soluble partitionate (CSF) and aqueous soluble partitionate AQSF respectively, for IC_{50} was 121.50±0.71, 112.50±1.14, 102.50±0.65 and 53.50±2.12. In comparison to the standard tert-butyl-1- hydroxytoluene (BHT) (IC_{50} 27.75±0.35) and ascorbic acid (ASA) (IC_{50} 5.95±0.21) in Table 1. The percent of inhibition versus Concentration was plotted in Figure 2.

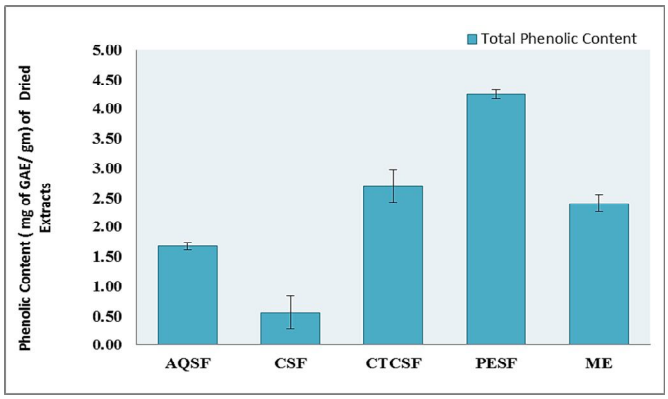

Fig 1: Total phenolic content various dried extracts

Table 1: Total Phenolic content of various dried extracts and IC_{50} Value of *In vitro* Free radical scavenging (DPPH) activity of *Pterospermum semisagittatum*

Name of the Samples	Total Phenolic Content (mg of GAE/gm of dried extract)	Free Radical Scavenging (DPPH) Value of IC_{50}
AQSF	1.67±0.06	27.75±1.06
CSF	0.56±0.29	53.50±2.12
CTCSF	2.70±0.27	112.50±1.14
PESF	4.25±0.08	102.50±0.65
ME	2.41±0.14	121.50±0.71
BHT	NA	27.75±0.35
ASA	NA	5.95±0.21

AQSF= aqueous soluble fraction,CSF= chloroform soluble fraction, CTSF= carbon tetrachloride soluble fraction, PESF= pet ether soluble fraction, ME= methanol extract, BHT = Butylated hydroxytoluene, ASA = Ascorbic Acid and NA = Not Analyzed. All calculation is performed with triplicate mean± Standard Deviation.

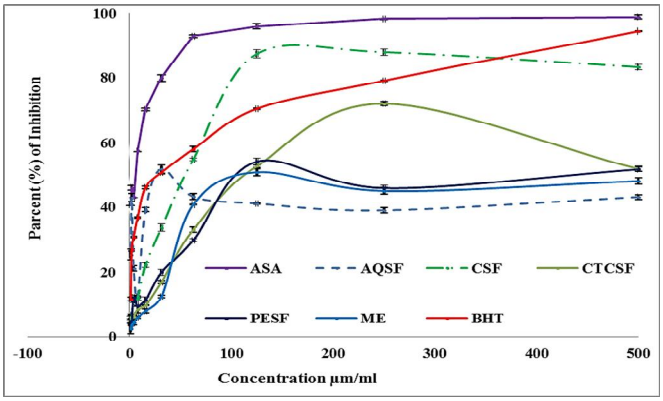

Fig 2: Comparative DPPH radical scavenging activity of different extractive of *Pterospermum semisagittatum* leaves along with BHT and Ascorbic acid including Mean ± Standard deviation

Table 2: Antimicrobial activity of test samples of *Pterospermum semisagittatum*

Test microorganisms	Diameter of zone of inhibition (mm)					
	AQSF	CSF	CTCSF	PESF	ME	Ciprofloxacin
Gram positive bacteria						
Bacillus cereus	-	10	-	-	-	40
Bacillus megaterium	-	14	-	-	-	50
Bacillus subtilis	-	8	-	-	-	36
Staphylococcus aureus	-	-	-	-	-	42
Sarcina lutea	-	10	-	-	-	42
Gram negative bacteria						
Escherichia coli	-	-	-	-	-	50
Pseudomonas aureus	-	-	-	-	-	49
Salmonella paratyphi	-	8	-	-	-	37
Salmonella typhi	-	12	-	-	-	35
Shigella boydii	-	-	-	-	-	45
Shigella dysenteriae	-	10	-		-	40
Vibrio mimicus	-	-	-	-	-	42
Vibrio parahaemolyticus	-	12	-	-	-	36
Fungi						
Candida albicans	-	-	-	-	-	35
Aspergillus niger	-	8	-	-	-	34
Saccharomyces cerevisiae	-	-	-	-	-	45

AQSF= aqueous soluble fraction, CSF= chloroform soluble fraction, CTSF= carbon tetrachloride soluble fraction, PESF= pet ether soluble fraction, ME= methanol extract

The exploratory antimicrobial activity of the extracts was determined at 400 µg/disc by the disc diffusion method against a number of Gram positive and Gram negative bacteria and fungi. The results are given in the Table 2.

During the measurement of zone of inhibition, only a chloroform soluble fraction has the slight amount of antimicrobial and antifungal activity. Highest amount of activity shown against *Bacillus megaterium* (14 mm), *Salmonella tophi* (12 mm),*Vibrio parahaemolyticus* (12mm), *Bacillus cereus* (10 mm), *Sarcina lutea* (10 mm), *Shigella dysenteriae* (10 mm), *Bacillus subtilis* (8 mm), *Salmonella paratyphi* (8 mm) and antifungal activity *Aspergillus niger* (8 mm) etc. But the comparative Standard ciprofloxacin is much higher active than *Pterospermum semisagittatum.*

4. Conclusion

The results of Phenolic content and Free radical scavenging (DPPH) were relatively significant. Further more focused research on this plant is recommended. Scientists can obtain more significant medicinal compounds from this plant.

5. Acknowledgement

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