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In vitro evaluation of antioxidant potentiality and estimation of total phenolic and flavonoid content of the whole stem of *Coffea benghalensis* B. Heyne ex Schult

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Abstract
This study was carried out for the first time to evaluate the antioxidant properties and total phenolic and flavonoid content of the methanolic extract along with its organic and aqueous soluble fractions of the whole stem of *Coffea benghalensis* growing in Bangladesh. For evaluation of antioxidant properties DPPH scavenging assay, ferric reducing capacity, hydroxyl radical scavenging assay and phospho molybdenum assay was used. In the DPPH free radical scavenging assay, the ethyl acetate soluble fraction of the crude methanol extract revealed the highest free radical and hydroxyl radical scavenging activity with IC_{50} value of 3.22 ± 0.17 µg/ml and 12.88 ± 0.305 µg/ml, respectively while the standard BHT was 4.39 ± 0.02 µg/ml and 9.38 ± 0.075 µg/ml, respectively. All the test samples and the standard BHT exhibited good linear relationship in the ferric reducing capacity and phospho molybdenum assay. Also, the test samples showed significant activities in the ferric reducing capacity and phospho molybdenum assay compared to the reference standard in a dose dependent manner. It was observed that the test samples contained considerable amount of bioactive compounds including total phenolic (ethyl acetate soluble fraction giving the highest 22.631 ± 0.085 GAE/gm of dried sample) and flavonoid (pet ether soluble fraction giving the highest 54.513 ± 1.500 CE/gm of dried sample) content. It can be concluded from the results that the crude methanolic extract along with it's four soluble fractions of the whole stem of *Coffea benghalensis* growing in Bangladesh are a good source of natural antioxidants.

Keywords: *Coffea benghalensis*, antioxidant, free radical scavenging, hydroxyl radical scavenging, phospho molybdenum assay

1. Introduction
Antioxidants are vital substances which possess the ability to protect the body from damage caused by free radical induced oxidative stress [1]. Over production of various forms of activated oxygen species, such as oxygen radicals and non-free radical species is considered to be the main contributor to oxidative stress, which has been linked to several diseases like atherosclerosis, cancer, and tissue damage in rheumatoid arthritis [2, 3]. Besides, hydroxyl radical is one of the most reactive oxygen species in the biological system. It reacts with cell membrane phospholipids polyunsaturated fatty acid moieties and causes damage to cell [4]. Synthetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), which are effective in their role as antioxidants, are commercially available and currently used in industrial processes. However, since suspected actions as promoters of carcinogenesis and other side effects have been reported, their use in food, cosmetic and pharmaceutical products has been decreasing [5-9].
Hence, there has been an upsurge of interest in naturally-occurring antioxidants from vegetables, fruits, flowers, leaves, oil-seeds, cereal crops, tree barks, roots, spices and herbs [10-13]. Numerous crude extracts and pure natural compounds from fruits were reported to have antioxidant and radical-scavenging activities. Within the antioxidant compounds, flavonoids and phenolics, with a large distribution in nature, have been studied [14]. Phenolics or polyphenols, including flavonoids have received considerable attention because of their physiological functions such as antioxidant, antimutagenic and antitumor activities [15]. Medicinal plant is any plant where one or more of its organs contains substances which can be used as therapeutic agents and which are precursors for synthesis and development of novel therapeutic agents. The tendency on the use of medicinal plants had yet been placed on the treatment rather than prevention of diseases [16]. Plants have been used for several years as a source of traditional medicine to treat various diseases and conditions [17].

Coffea benghalensis B. Heyne ex Schult. is a deciduous shrub, commonly known as bonnyo koffee belonging to Rubiaceae family. In Nepal the flowers are used for excessive bleeding during menstruation [18]. Also, the fruit part of *C. benghalensis* showed antibacterial activities against *Proteus vulgaris*, *Escherichia coli*, *Vibrio cholerae*, *Salmonella typhi* and *Streptococcus aureus* [19]. The native distribution of this plant has been documented in Bangladesh (Sylhet to Chittagong), India (Arunachal Pradesh, Assam, Bengal, Meghalaya, Orissa, Rajasthan and Sikkim), East Himalaya, Bhutan, Myanmar, Nepal, Thailand, Vietnam [20]. The leaf, pericarp and seed part of the plant exhibited nine polyphenolic compounds of which caffeic acid, chlorogenic acid, ferulic acid, *p*-coumaric acid and sinapic acid are phenolic acids and quercetin, isoquercitrin, rutin and kaempferol are flavonols [19]. Also from the leaves a cafestol (bengalensol) was identified [21]. The fruit part of *C. benghalensis* showed antibacterial activities against *Proteus vulgaris*, *Escherichia coli*, *Vibrio cholerae*, *Salmonella typhi* and *Streptococcus aureus* [19]. Literature survey showed that there are no phytochemical, biochemical or pharmacological information concerning the whole stem of *Coffea benghalensis* B. Heyne ex Schult. So, as part of our continuing studies on medicinal plants of Bangladesh [22] especially *Coffea benghalensis* B. Heyne ex Schult. growing in Bangladesh we investigated the antioxidant potential in forms of free radical scavenging activity, ferric reducing capacity, hydroxyl radical scavenging activity and total antioxidant capacity (Phosphomolybdenum assay), also total phenolic and flavonoid content of the whole stem of *C. benghalensis* was done for the first time, and we, herein, report the results of our preliminary studies.

2. Materials and Methods

2.1 Chemicals

Most of the chemicals and reagents like Folin-ciocalteu reagent, sodium phosphate, ammonium molybdate, ferric chloride, methanol, DPPH, BHT, EDTA, $FeSO_4.7H_2O$, aluminium chloride were purchased from Sigma chemical company, USA. Potassium ferricyanide, trichloro acetic acid, sulfuric acid, sodium carbonate and hydrogen peroxide were purchased from Merck, Germany. But phosphate buffer, 2-deoxy-D-ribose, thiobarbituric acid were bought from Sigma-Aldrich, Japan. Gallic acid and catechin were obtained from Wako pure chemicals Ltd., Japan. All the chemicals and solvents used were of analytical reagent grade and all are commercially available.

2.2 Plant Materials

The stems of *C. benghalensis* were collected during the rainy monsoon season from Modhupur forest, Tangail, Bangladesh in 2018. The plant was authenticated at the Bangladesh National Herbarium, where a voucher specimen has been maintained representing this collection (DACB Accession No. 45789). The stems were sun dried for ten days after collection and proper washing. Then the sun dried stems were cut into small pieces, cleaned and oven dried for 48 hours at a considerably low temperature (not more than 30 °C) and coarsely powdered.

2.3 Extraction

In 5L methanol 1000 gm of the coarsely powdered plant material was soaked for about 15 days with occasional shaking and stirring. It was at first filtered through a fresh cotton plug and finally with filter paper (Whatman No. 1). The filtrate was then concentrated to dryness, in vacuum by using rotary evaporator at 40 °C temperature to render the crude methanolic extract (21 gm).

The modified Kupchan partitioning protocol [23] was used for the partitioning of an aliquot of the concentrated methanol extract (CME). The consequent partitionates were dried off to yield pet-ether (PESF), chloroform (CSF), ethyl acetate (EASF),and aqueous (AQSF) soluble materials (Table 1). The residues were then stored in a cool dry place until further use.

Table 1: Modified Kupchan partitionates of *C. benghalensis* whole stem

Crude extract/ Soluble fractions	Weight (gm)
CME	10
PESF	1.47
CSF	4.21
EASF	0.14
AQSF	4.15

2.4 Antioxidant Activity

2.4.1 DPPH free radical scavenging assay

Following the method developed by Braca *et al.*, 2001 [24] the antioxidant activity of the test samples was assessed by evaluating the scavenging activities of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical by using synthetic antioxidant, butylated hydroxytoluene (BHT)as reference standards.according to this method absorbance was taken at 517 nm because the DPPH radical contains an odd electron, which is responsible for the absorbance and also for the visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the change in absorbance and % of scavenging activity is calculated. According to this method 0.1 ml of each sample (CME, PESF, CHSF, EASF and AQSF) was added to 3 ml of a 0.004% methanol solution of DPPH and five different concentrations (25, 50, 100, 200 and 400 µg/ml) were made. Absorbance was taken at 517 nm after 30 min and the percentage inhibition activity was calculated by using the following equation-

$$(I\ \%) = (1 - A_{sample} / A_{blank}) \times 100$$

where, A_{blank} is the absorbance of the control (containing all reagents except the test material and A_{sample} is the absorbance of the sample extractive.

Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted inhibition percentage inhibition against all the extractive and/or standard concentration.

2.4.2 Ferric Reducing Capacity Assay

Ferric reducing capacity assay is based on reduction of a colorless Fe3+-TPTZ complex into intense blue Fe2+-TPTZ once it interacts with a potential antioxidant.

Oyaizu *et al.* (1986) [25] method was used to assay the reducing power of the crude methanolic extract and its four soluble fractions. Here the transformation of Fe^{3+} to Fe^{2+} was analyzed using BHT as standard.

In this method 0.25 ml of each of the samples (all the extractives and standard solution), at various concentrations (25-400 µg/ml) was taken in test tube. Then phosphate buffer (0.625 ml, 0.2 M, pH 6.6) and potassium ferricyanide [K_3Fe

$(CN)_6$] (0.625 ml,1%) was added in each test tube. At 50 °C the mixture was then incubated at 50 °C 20 min. Instantly, the mixture solution was cooled, mixed with trichloro acetic acid (0.625 ml, 10%) and centrifugated at 3000 rpm for 10 min. In the next step, the upper layer (1.8 ml) was mixed with distilled water (1.8 ml) and which was then mixed with ferric chloride [$FeCl_3$] (0.36 ml, 1% w/v) and the absorbance was measured at 700 nm. The reducing power capacity was determined from the absorbance value of the test sample, higher the absorbance value indicated stronger reducing power. BHT was used as the reference and phosphate buffer (pH 6.6) was used as blank solution.

2.4.3 Hydroxyl radical scavenging assay

The hydroxyl radical scavenging activity of *C. benghalensis* extracts were determined by the method described by Halliwell and Gutteridge [26] with some modification [27]. Here the ability of the different soluble fractions and crude methanolic extract of the stems *C. benghalensis* to scavenge the hydroxyl radicals generated by the Fe^{3+} ascorbate-EDTA-H_2O_2 system (Fenton reaction) was analyzed. The assay was performed by adding 0.1 mL EDTA, 0.01 mL of $FeCl_3$,0.1 mL H_2O_2, 0.36 mL of deoxyribose, 1.0 mL of the sample solutions of different concentration (25, 50, 100, 200 & 400 µg/mL) dissolved in distilled water, 0.33 mL of phosphate buffer (50 mM, pH 7.4), 0.1 mL of ascorbic acid in sequence. The mixture was then incubated at 37 ℃ for 1 hour. 1 ml of 0.5% thiobarbituric acid (in 0.025 M NaOH containing 0.025% BHA) and 1 ml of 10% trichloroacetic acid were added to it and further incubated at 100 ºC for 20 minutes. Finally the reaction mixture was cooled on ice and centrifuged at 5000 rpm for 15 min. Absorbance of supernatant was measured at 532 nm against a blank containing deoxy-D-ribose and buffer. The percentage of scavenging activity was calculated by:

$$\% \text{ scavenging} = [(A_0-A_1)/A_0]\times 100$$

where A_0 = the absorbance of the control and A_1 = the absorbance of the sample/standard BHT. The percentage of scavenging was plotted against concentration and IC_{50} was calculated from the graph.

2.4.4 Phosphomolybdenum assay

The antioxidant activity of the crude methanolic extract and its four soluble fractions were evaluated by the phosphomolybdenum method with some modifications [28]. The method was based on the reduction of Mo (VI) to Mo (V) by the extracts and subsequent formation of a green phosphate-Mo (V) complex in acidic condition. The phosphomolybdate solution was prepared by adding 1 ml each of 0.6 M H_2SO_4, 28 mM sodium phosphate and 4 mM ammonium molybdate in 50 ml distilled. 0.5 ml of each of the crude methanolic extract of *C. benghalensis* and its four soluble fractions/standard in different concentration ranging from 25 µl/mg to 500 µl/mg were added to each test tube individually containing 3 ml of distilled water and 1 ml of molybdate reagent solution. At this point the tubes were then kept incubated at 95 ℃ for 90 min.The temperature of the test tubes were then brought to room temperature which took around 20-30 min and the absorbance measured at 695 nm. Mean values from three independent samples were calculated for each each. BHT was used as positive reference standard. The antioxidant capacity was expressed as the number of milligrams equivalent of BHT per gram of dried extract. Increased total antioxidant capacity was indicated at increased absorbance of the reaction mixture. The experiment was repeated three times at each concentration.

2.5 The amount of phenolic compounds and flavonoids

Plant polyphenols, a diverse group of phenolic compounds possess an ideal structural chemistry for free radical scavenging activity [29]. Total phenolic content of the test samples of stem of *C. benghalensis* (CME, PESF, CHSF, EASF & AQSF) was ascertained by applying the standard spectrophotometric method as set forth by Skerget *et al.*, 2005 [30], where gallic acid is used as standard and Folin-Ciocalteu reagent as oxidizing agent. The phenolic contents of the sample were expressed as mg of GAE/gm of dried extract.

The content of total flavonoid from the different soluble fractions and crude methanolic extract of the stems of *C. benghalensis* was assayed by aluminium trichloride colorimetric method where catechin is used as standard [31] and the results were expressed as mg of catechin equivalent (CE)/g of dried extract.

2.6 Statistical analysis: All the values in the test are expressed as mean ± standard deviation (SD).

3. Results

The current study was taken over to evaluate the antioxidant activities of the crude methanolic extract (CME) and its different soluble fractions (PESF, CSF, EASF & AQSF) of the whole stems of Bangladeshi *C. benghalensis* species. The results of the different antioxidant assays are given in Tables 2 & 3 and Figures 1 to 4.

As DPPH is the most stable radical and due to its ease and availability it has been widely used for free radical-scavenging assessments. Concentration of the test samples between 25-400 µg/ml remarkably increased the inhibitory activity (Figure 1) e.i the percentage (%) scavenging of DPPH free radical was found to be concentration dependent. The EASF exhibited strongest IC_{50} value of 3.22 ± 0.17 µg/ml while for standard BHT it was found to be 4.39 ± 0.02 µg/ml, while the weakest scavenger was PESF with IC_{50} value of 9.78 ± 0.11 µg/ml. Thus, by comparison with the standard BHT we can say that not only EASF has more potential radical scavenging activity than the standard, but the weakest scavenger in this study has also remarkable scavenging activity (Table 2).

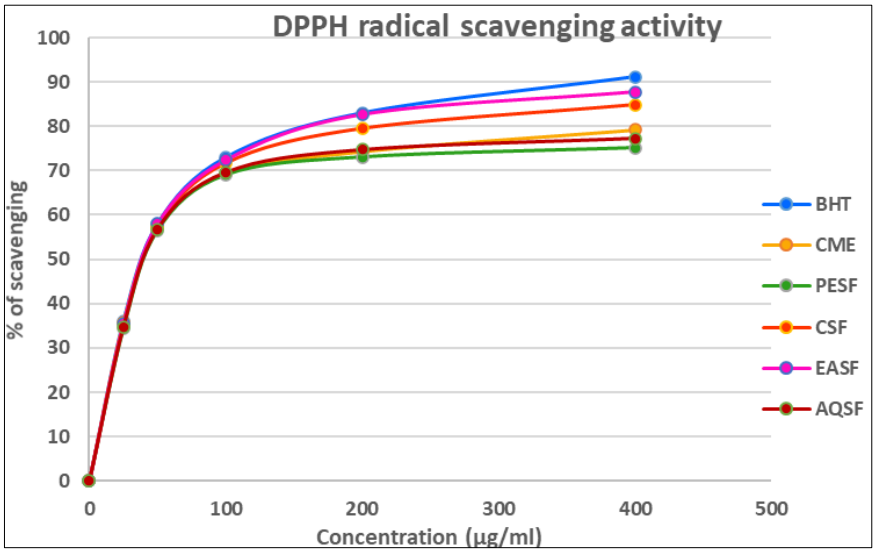

Fig 1: DPPH free radical scavenging activity of CME, PESF, CSF, EASF, AQSF and standard BHT

Table 2: Free radical scavenging, ferric reducing capacity, hydroxyl radical scavenging and total antioxidant activities of *C. benghalensis*

Plant	DPPH free radical scavenging activity (IC_{50} µg/ml)	Ferric reducing capacity (% of inhibition at 400 µg/ml)	Hydroxyl radical scavenging activity (IC_{50} µg/ml)	Total antioxidant capacity (% of inhibition at 400 µg/ml)
Stem				
CME	7.21 ± 0.05	0.764 ± 0.08	28.25 ± 0.215	1.481 ± 0.23
PESF	9.78 ± 0.11	0.432 ± 0.02	33.37 ± 0.210	1.552 ± 0.08
CSF	6.01 ± 0.00	1.143 ± 0.07	15.94 ± 0.560	0.540 ± 0.10
EASF	3.22 ± 0.17	1.455 ± 0.08	12.88 ± 0.305	0.746 ± 0.07
AQSF	7.59 ± 0.25	0.967 ± 0.11	25.44 ± 0.045	0.258 ± 0.09
Standard				
BHT	4.39 ± 0.02	2.533 ± 0.05	9.38 ± 0.075	1.578 ± 0.32

The reductants' reducing power is mostly related by its antioxidant action by breaking the free radical chains. This is done by donating a hydrogen atom. The antioxidant samples capacity to reduce Fe^{3+}/ferricyanide complex to the Fe^{2+}/ferrous form indicated the presence of reductants. Therefore, the ferric reducing power of the samples were monitored by measuring the formation of Perl's Prussian blue at 700 nm [32] as shown in Table 2. From Figure 2 it was observed that the different test samples' (CME, PESF, CSF, EASF & AQSF) electron donating capacity was concentration dependant and demonstrated good linear relation in both standard (R^2 = 0.9809) and test samples' (CME, PESF, CSF, EASF & AQSF) (R^2 = 0.9721, 0.9652, 0.9965, 0.9833 and 0.9603, respectively.) (Figure 2). At the concentration of 400 µg/ml EASF exhibited the strongest activity with an absorbance of 1.455 ± 0.08, which was close to the activity of the reference standard BHT that gave an absorbance of 2.533 ± 0.05 at the same concentration (Table 2).

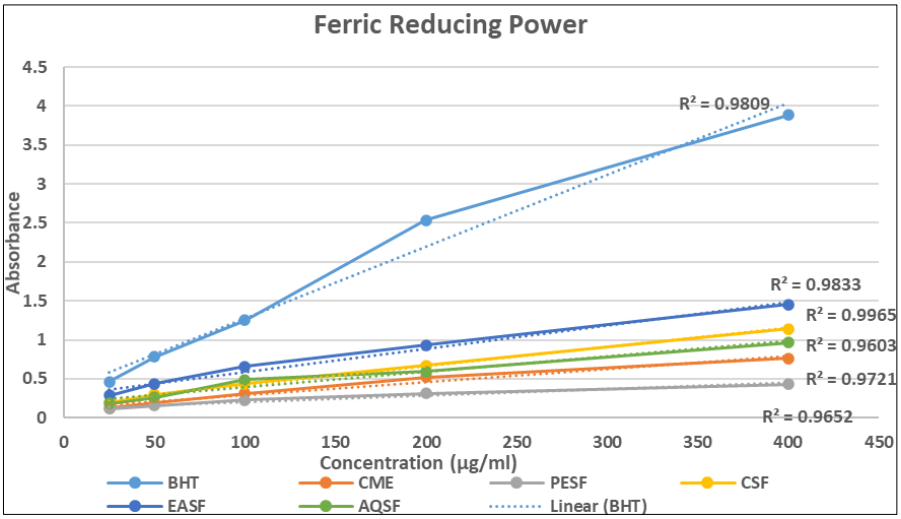

Fig 2: Ferric reducing capacity of CME, PESF, CSF, EASF, AQSF and standard BHT

Major pathological conditions like cancer, neurodegenerative disorders, etc. may generally be caused by ROS [33]. The mostly formed ROS are oxygen radicals, such as hydroxyl radicals and superoxide, and non-free radicals, such as hydrogen peroxide and singlet oxygen [34]. Among these, hydroxyl radical is the most reactive and induces severe

damage to adjacent biological molecules. Thus, the hydroxyl radical scavenging activity of the crude methanolic extract (CME) and its four soluble fractions (PESF, CSF, EASF & AQSF) was investigated using the 2-deoxyribose method. All the test samples showed considerable scavenging properties against hydroxyl radicals, and the inhibition percentage was proportional to the concentration of each compound (Figure 3). As seen in Table 2 the highest hydroxyl scavenging activity was exhibited by EASF with IC_{50} value of 12.88 ± 0.305, followed by CSF (IC_{50} 15.94 ± 0.56), no tested compound showed better antioxidative activity than the positive control (BHT). Our results showed that the IC_{50} value of PESF was about 3.5 fold than that of BHT.

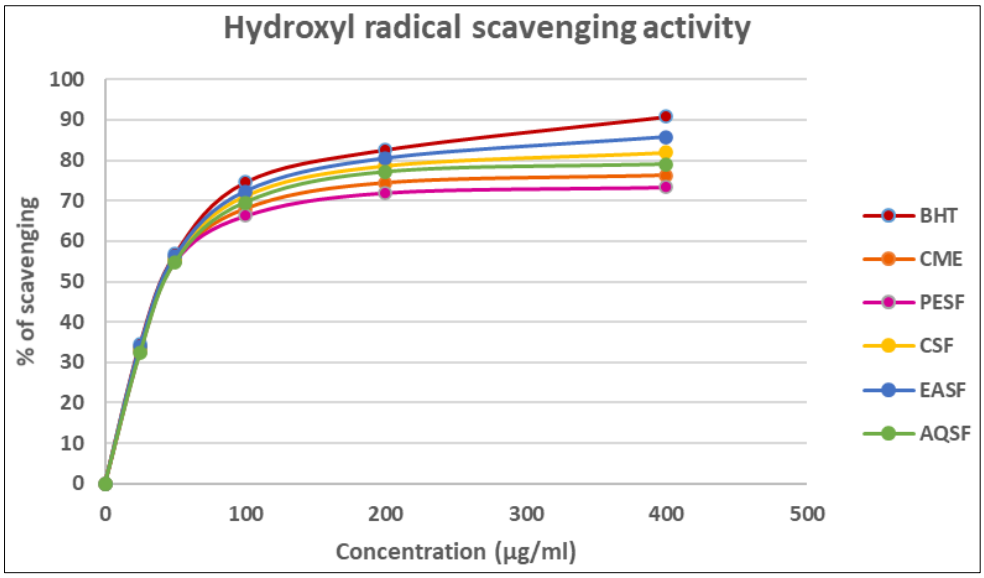

Fig 3: Hydroxyl radical scavenging activity of CME, PESF, CSF, EASF, AQSF and standard BHT.

The reduction of Mo(VI) to Mo(V) and consequent formation of a green phosphate/Mo(V) complex at acidic pH indicated the total antioxidant capacity of the test samples (CME, PESF, CSF, EASF & AQSF). This assay is called total antioxidant capacity (TAC) as it evaluates both water soluble and fat soluble antioxidants [35]. In this assay, BHT (synthetic antioxidant) is used as reference standard. As shown in Figure 4, we can see that the test samples showed antioxidant activity in a concentration dependant manner, i.e as the concentration increased absorbance was higher. Highest absorbance, at 400 µg/ml was given by PESF (1.552 ± 0.08) and lowest was given by AQSF (0.258 ± 0.09) which was nearly 6 fold lower than the reference standard BHT (1.578 ± 0.32). The TAC at 400 µg/ml was found to decrease in order of PESF > CME > EASF > CSF > AQSF, with absorbance 1.552 ± 0.08, 1.481 ± 0.23, 0.746 ± 0.07, 0.540 ± 0.10 and 0.258 ± 0.09, respectively.

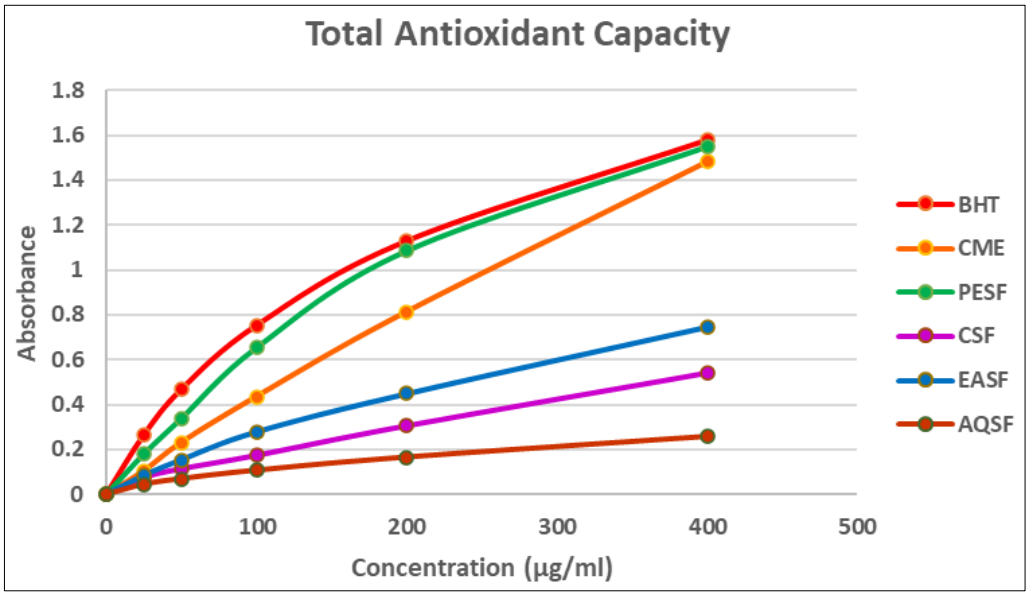

Fig 4: Total antioxidant capacity of CME, PESF, CSF, EASF, AQSF and standard BHT.

It is well known that phenolic compounds are potential antioxidants and free radical-scavengers; hence, there should be a close correlation between the content of phenolic compounds and antioxidant activity [36]. In this study, the crude methanolic extract (CME) of *C. benghalensis* and its four soluble fractions (PESF, CSF, EASF & AQSF) were

analyzed for its TP content. The TP content varied in the different extracts and ranged from 6 to 23 mg GAE/gm of dried sample (Table 3). EASF had the highest TP content (22.631 ± 0.085 GAE/gm of dried sample), followed by CSF (10.566 ± 0.081 GAE/ gm of dried sample), exhibiting that EASF had extensive phenolic content in comparison to the other test samples. The TP contents were in the following sequence: EASF > CSF > CME > AQSF> PESF. The total phenolic content was calculated using the standard curve of gallic acid ($y = 0.102x - 0.0268$; $R^2 = 0.9996$).

Table 3: Total phenolic content and total flavonoid content of *C. benghalensis*

Sample	Total phenolic content (GAE/gm of dried sample)	Total flavonoid content (CE/gm of dried sample)
Stem		
CME	9.331 ± 0.072	45.663 ± 0.775
PESF	6.376 ± 0.042	54.513 ± 1.500
CSF	10.566 ± 0.081	41.618 ± 0.523
EASF	22.631 ± 0.085	43.321 ± 1.125
AQSF	6.873 ± 0.192	40.4621.118

All the test samples (CME, PESF, CSF, EASF & AQSF) demonstrated fairly good flavonoid content with PESF (54.513 ± 1.500 CE/gm of dried sample) having highest total flavonoid content and AQSF contained the lowest flavonoid of 40.462 ± 1.118 CE/gm of dried sample (Table 3). Total content of flavonoid of the test samples were in the order of PESF > CME > EASF > CSF > AQSF. The total flavonoid content was calculated using the standard curve of catechin ($y = 0.027x - 0.053$; $R^2 = 0.999$).

4. Discussions

In living organisms for the production of energy to fuel biological processes oxidation is essential. Yet, oxygen-centered free radicals and other reactive oxygen species that are continuously produced *in vivo*, result in cell death and tissue damage. Oxidative damage caused by free radicals may be related to aging and diseases, such as atherosclerosis, diabetes, cancer and cirrhosis [37]. The DPPH assay measured the antioxidant activity of tested substances. The scavenging effect of *C. benghalensis* crude methanol extract (CME) and it's four soluble fractions (PESF, CSF, EASF & AQSF) and standard BHT were thus assessed and expressed in terms of the concentration of the test samples necessary to reduce by 50% the initial quantity of DPPH. According to Jun *et al*., 2003 [38] and Mustarichie *et al*., 2017 [39] antioxidant activity of these test samples were categorized as very powerful, because IC_{50} of all the test samples were less than 50 µg/mL as shown in Table 2.

In the ferric reducing capacity assay, the yellow colour test solution changed to green and blue depending on the reduction capacity of the crude test samples [40, 41] [**39**, **40**]. The presence of reductants in the test solution reduced Fe^{3+} to Fe^{2+}, which can be monitored by measurement of Perl's Prussian blue colour at 700 nm [42] [**41**]. The transformation ability of compounds from Fe^{3+}/ferricyanide complex to Fe^{2+}/ferrous form acts as a potential indicator for antioxidant activity [43] [**38**]. According to Benzie *et al.*, 1996 [44] [**42**] the ferric reducing capacity assay of the test samples was convenient, reproducible and linearly concentration-dependent.

The hydroxyl racidal has a high and indiscriminate activity and can slowly cause severe damage to susceptible biomolecules. Hydroxyl radicals can be produced in cells by a variety of processes such as phagocytosis [45], prostaglandin biosynthesis especially during the transformation of PGG_2 to PGH_2 [46], decomposition of lipid hydroperoxides [47], etc. It is generally proposed that such a radical could be originated by Fenton-type reaction [48]. Hydroxyl radicals, being extremely reactive species, serve as both a primary toxicant and as a source of secondary toxicants [49]. They are reported to mediate the lethal injury in cultured hepatocytes [50] and contribute to significant biological effects such as carcinogenesis, mutagenesis and cytotoxity [51]. The lowest IC_{50} means the highest hydroxyl radical scavenging capacity. Thus the IC_{50} were used to determine hydroxyl radical scavenging capacity of the test samples and were compared to standard BHT.

Phosphomolybdenum assay measure reduction of Mo (VI) to Mo (V) and subsequent formation of a green phosphate-Mo (V) complex at acidic pH [29]. Molybdenum is the active side of xanthine oxidase, an enzyme that produces free radicals [52]. So as the concentration of the test samples increased absorbance was higher indicating that antioxidant activity is concentration dependant.

Flavonoids and phenols are secondary metabolites with free radical scavenging abilities that are widely distributed in fruits, leaves, bark and other parts of plants [53]. Natural antioxidants such as flavonoids and phenolic compounds are believed to possess antioxidant properties due to their reducing and chelating capabilities [54].

Due to high specificity for polyphenolic compounds the total phenolic content of the test samples was determined by Folin Ciocalteu reagent as it does not interact with the other phytochemicals due to complex formation. The results for DPPH are in agreement with the polyphenol (phenols and flavonoids) contents determined for each sample. Our findings in this assay implied that all the test samples were rich in phenolic and flavonoid contents which are the major contributor to scavenge the free radicals in oxidation pathways.

5. Conclusion

Our current study indicated that the crude methanolic extract along with it's four soluble fractions of the whole stem of Bangladeshi *Coffea benghalensis* B. Heyne ex. Schult contains significant quantity of total polyphenols and flavonoids and exhibited good antioxidant activity by effectively scavenging various free radicals. This antioxidant activity might be due to the synergistic actions of bioactive compounds present in the plant. However, it is still unclear which components are playing vital roles for this activity. Nevertheless, this scientific information can serve as an important platform for the development of safe and effective natural medicine. So, further investigation is underway to isolate the promising bioactive constituents.

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