Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2021; 10(2): 40-45
Received: 10-01-2021
Accepted: 19-02-2021

Rifat ARA Rima
Department of Pharmacy, Faculty of Science, University of Rajshahi, Rajshahi, Bangladesh

Shoriful Islam Sagor
Department of Pharmacy, Faculty of Science, University of Rajshahi, Rajshahi, Bangladesh

Adeeba Anjum
Associate Professor, Department of Pharmacy, University of Rajshahi, Rajshahi, Bangladesh

Corresponding Author:
Adeeba Anjum
Associate Professor, Department of Pharmacy, University of Rajshahi, Rajshahi, Bangladesh

In vitro antioxidant activities of the roots of *Coffea benghalensis* B Heyne ex Schult. Growing in Bangladesh

Rifat Ara Rima, Shoriful Islam Sagor and Adeeba Anjum

Abstract
In the present study, antioxidant potential of the crude methanol extract along with its *n*-hexane, chloroform, ethyl acetate and aqueous soluble fractions of the roots of *Coffea benghalensis* growing in Bangladesh was assessed by using DPPH scavenging assay, reducing power, hydroxyl radical scavenging assay and total antioxidant capacity. In DPPH scavenging assay the IC_{50} value of the ethyl acetate soluble fraction was found to be 47.56 µg/ml while the reference standard ascorbic acid was 1.42 µg/ml. The reducing power showed good linear relations between the standard ascorbic acid and all the test samples. Higher hydroxyl radical activity was shown by the *n*-hexane soluble fraction with IC_{50} value 58.58 µg/ml whereas the standard catechin was 89.61 µg/ml. The extracts showed significant activities in total the antioxidant assay compared to the reference standard in a dose dependent manner. Also total phenolic and flavonoid content was evaluated which exhibited remarkable values.

Keywords: *Coffea benghalensis*, antioxidant, free radical scavenging, total antioxidant capacity

1. Introduction
Antioxidants interact with and stabilize free radicals and may prevent some of the damage free radicals might otherwise cause. Free radical damage may lead to cancer. Examples of antioxidants include beta-carotene, lycopene, vitamins C, E, A and other substances [1]. An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reactions by being oxidized themselves. As a result, antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols [2]. Plants have long been a source of exogenous (i.e., dietary) antioxidants. It is believed that two-thirds of the world's plant species have medicinal importance, and almost all of these have excellent antioxidant potential [3]. The interest in the exogenous plant antioxidants was first evoked by the discovery and subsequent isolation of ascorbic acid from plants [4]. Since then, the antioxidant potential of plants has received a great deal of attention because increased oxidative stress has been identified as a major causative factor in the development and progression of several life threatening diseases, including neurodegenerative and cardiovascular disease. In addition, supplementation with exogenous antioxidants or boosting of endogenous antioxidant defenses of the body has been found to be a promising method of countering the undesirable effects of oxidative stress [5].
The current research involves the antioxidant activities of locally known Bonnyo kofee, *Coffea benghalensis* B. Heyne ex Schult. of Rubiaceae family. The plant is a deciduous shrub, up to 2-3 m tall [6], whose native distribution has been documented in Bangladesh (Sylhet to Chittagong), India (Arunachal Pradesh, Assam, Bengal, Meghalaya, Orissa, Rajasthan and Sikkim), East Himalaya, Bhutan, Myanmar, Nepal, Thailand and Vietnam [7]. About nine polyphenolic compounds was found in the leaf, pericarp and seed. Of the nine compounds, five are phenolic acids (caffeic acid, chlorogenic acid, ferulic acid, *p*-coumaric acid and sinapic acid) and other 4 compounds identified as flavonols (quercetin, isoquercitrin, rutin and kaempferol) [8]. A cafestol (Bengalensol) was identified from the leaves [9]. The flowers of *C. benghalensis* were used in Nepal for excessive bleeding during menstruation [10]. Fruit part of *C. benghalensis* showed antibacterial activities against *Proteus vulgaris*, *Escherichia coli*, *Vibrio cholerae*, *Salmonella typhi* and *Streptococcus aureus* [11].

2. Materials and Methods
2.1. Plant materials
The roots of *C. benghalensis* were collected from Modhupur forest, Tangail, Bangladesh

in August 2017. The plant was identified at the Bangladesh National Herbarium, where a voucher specimen has been maintained representing this collection (DACB Accession No. 45789). After proper washing the root were sun dried for seven days. The sun dried roots were cut into small pieces, cleaned, oven dried for 48 hours at considerably low temperature (not more than 30 °C) and pulverized.

2.2. Extraction

1000 gm of the powdered material was soaked in methanol and kept for a period of 13 days accompanying occasional shaking and stirring. The whole mixture was then filtered through a fresh cotton plug and finally using filter paper (Whatman No. 1). The volume of the filtrate was then concentrated using rotary evaporator under reduced pressure at 40 °C temperature to obtain the crude methanolic extract. A portion (10 g) of the concentrated methanol extract (Me) of roots of *C. benghalensis* was fractionated by the modified Kupchan partitioning method [12] into *n*-hexane (HSF), chloroform (CHSF), ethyl acetate (EASF) and aqueous (AQSF) soluble fractions and the yields are summarized in Table 1.

Table 1: Kupchan partitioning of a portion of the crude extract (10 gm) of the roots of *C. benghalensis* growing in Bangladesh

Soluble fraction	Weight (gm)
n-Hexane (HSF)	3.39
Chloroform (CHSF)	2.29
Ethyl acetate (EASF)	0.21
Aqueous (AQSF)	3.11

2.3. Antioxidant activity

2.3.1. DPPH radical scavenging activity

The methanolic extract of the roots of *C. benghalensis* along with its *n*-hexane (HSF), chloroform (CHSF), ethyl acetate (EASF) and aqueous (AQSF) soluble fractions were tested for their free radical scavenging activity based on the scavenging activity of the stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) was determined by the method of Braca *et al*.,(2001) [13] Two ml of a methanol solution of the samples (all the extractives and/or standard) at different concentrations (100 µg/ml to 6.25 µg/ml) were mixed with 3.0 ml of a DPPH methanol solution (20 µg/ml). After 30 mins of reaction at room temperature in dark place the absorbance was measured at 517 nm against methanol as blank by using a spetrophotometer. Then inhibition of free radical DPPH in percent (I%) was calculated as follows:

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

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.

2.3.2. Reducing power

Reducing power was investigated by using Oyaizu, 1986 method [14]. Here 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 0.625 ml phosphate buffer (0.2 M, pH 6.6) and 0.625 ml 1% potassium ferricyanide was added in each test tube and the mixture was then incubated at 50 °C for 20 min. The mixture solution was cooled rapidly, mixed with 0.625 ml of trichloro acetic acid (10%) and centrifuged at 3000 rpm for 10 min. The supernatant (1.8 ml) was then mixed with 1.8 ml distilled water and 0.36 ml of 1% ferric chloride ($FeCl_3$). The supernatant (1.8 ml) was then mixed with distilled water (1.8 ml) and $FeCl_3$ (0.36 ml, 0.1% 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. Ascorbic acid was used as the reference and phosphate buffer (pH 6.6) was used as blank solution.

2.3.3. Hydroxyl radical scavenging assay

The hydroxyl radical scavenging assay was determined by the method of Halliwell *et al.,* (1987) [15]. Here the ability of the different extractives of *C. benghalensis* to scavenge the hydroxyl radicals generated by the Fe^{3+} ascorbate-EDTA-H_2O_2 system (Fenton reaction). In this method ascorbic acid-iron-EDTA is the hydroxyl generating system. In the Fenton reaction mixture the final volume of 1 ml of the reaction mixture consists of 100 µl of 2-deoxy-D-ribose (28 mM in 20 mM KH_2PO_4-KOH buffer, pH 7.4), 500 µl of samples (different concentrations), 100 µl of EDTA (1.04 mM), 100 µl of 200 µM $FeCl_3$ (1:1 v/v), 100 µl H_2O_2 (1 mM) and 100 µl ascorbic acid (1 mM) was incubated at 37 ºC for 1 hour. 1 ml of thiobarbituric acid (1%) and 1 ml of trichloroacetic acid (10%) were added to it and further incubated at 100 ºC for 20 minutes. After cooling, absorbance 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.

2.3.4. *Phosphomolybdenum assay*

The total antioxidant activity of the plant extracts was evaluated by the phosphomolybdenum method with some modifications [16] 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. 0.5 ml of each of the samples/standard at different concentrations (6.5-100 µg/ml) was mixed with 3 ml of a reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4mM ammonium molybdate), and the reaction mixture was incubated at 95 ºC for 90 minutes. Then the absorbance of the solution was measured at 695 nm using a UV-visible spectrophotometer against a blank solution. after cooling to room temperature. Here, ascorbic acid was used as standard and the blank solution contained methanol in place of sample solution. The antioxidant capacity was expressed as the number of milligrams equivalent of ascorbic acid 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.

2.4. Total phenol content

Total phenolic content of methanol extractives of root of *C. benghalensis* along with their *n*-hexane, chloroform, ethyl acetate and aqueous soluble fractions were measured by employing the method described by Skerget *et al*., 2005 [17]

involving Folin-Ciocalteu reagent as an oxidizing agent and gallic acid as a standard. To 0.5 ml of extract or standard solution in water was added to 2.5 ml of Folin-Ciocalteu reagent (diluted 10 times with water) and 2.0 ml of sodium carbonate (7.5% w/v) solution were added. The mixture was then incubated for 20 minutes at room temperature. After 20 minutes, the absorbance was measured at 760 nm using a visible spectrophotometer. Total phenolics were quantified by calibration curve obtained from measuring the absorbance values from known concentrations of gallic acid (0-100 µg/ml). The phenolic contents of the sample were expressed as gm of GAE (gallic acid equivalent) / 100 gm of the dried extract.

2.5. Total flavonoids content

Different soluble fractions of the roots of *C. benghalensis* was tested for its total flavonoid content by aluminium trichloride colorimetric method where catechin is used as standard [18]. 3 ml of methanol was added to 1 ml of the plant extract. Then 0.2 ml 10% $AlCl_3$, 0.2 ml 1 M potassium acetate and 5.6 ml of distilled water was added to it. For completion of the reaction mixture it was incubated for 30 minutes at room temperature. Finally, the absorbance of the mixture was measured at 420 nm. Catechin was used as standard and the results were expressed as mg of catechin equivalent (CE)/g of dried extract.

3. Results

The current study was undertaken to assess the antioxidant activities of the crude methanolic extract and its different soluble fractions of the roots of Bangladeshi *C. benghalensis* species. The results of the different antioxidant assays are given in Tables 2 to 5 and Figures 1 to 4. Comparison of DPPH radical scavenging activity of the crude methanolic extract and its different soluble fractions are illustrated in Figure 1. Lower IC_{50} value corresponds to a higher free radical scavenging activity of the plant extracts [19].The ethyl acetate soluble fraction of the root of *C. benghalensis* showed highest free radical scavenging activity having IC_{50} value of 47.56 µg/ml and lowest activity was shown by the chloroform soluble fraction being 137.986 µg/ml (Table 2). The other soluble fractions of the roots of the plant demonstrated low to moderate level of free radical scavenging activity with the IC_{50} value ranging from 110 µg/ml to 45 µg/ml, as compared to the standard, i.e. *tert*butyl-1-hydroxytoluene (BHT), (IC_{50} = 29.493 µg/ml).

Table 2: Free radical scavenging activity (IC_{50} in µg/ml) of extractives of roots of C. benghalensis growing in Bangladesh

Test material	IC_{50} (µg/ml)
Me	112.301 ± 1.572
HSF	76.405 ± 1.709
CHSF	137.986 ± 2.50
EASF	47.560 ± 0.220
AQSF	115.476 ± 1.21
BHT	29.493 ± 0.761

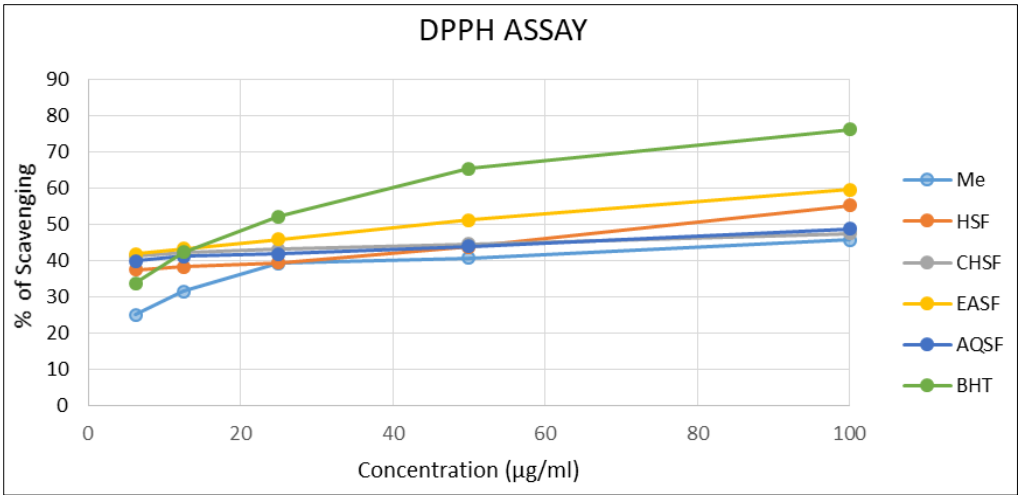

Fig 1: Comparison of DPPH radical scavenging activity of the crude methanolic extract and its different soluble fractions of *C. benghalensis*

The antioxidant activity of the different extractives was also measured by ferric reducing antioxidant power (FRAP). This method is based on the principle that substances, which have reduction potential, react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride to form ferric–ferrous complex that has an absorption maximum at 700 nm. The reducing power of the methanol extract along with its four different soluble fractions and standard (Ascorbic acid) increases with the increase in amount of sample and standard concentrations (Table 3). The reducing power shows good linear relation between the standard (R^2 = 0.9956) and the methanol extract along with its *n*-hexane, chloroform, ethyl acetate and aqueous soluble fractions (R^2 = 0.9972, 0.9904, 0.9966, 0.9917 and 0.987, respectively.) (Figure 2). The chloroform soluble fraction showed the highest activity with an absorbance of 0.348 at 100 µg/ml concentration, which was close to the activity of the reference standard ascorbic acid that gave an absorbance of 0.471 at the same concentration (Table 3)

Table 3: Reducing power activity of the methanolic extract and its different soluble fractions of *C. benghalensis*

Concentration (µg/ml)	Ascorbic acid	Me	HSF	CHSF	EASF	AQSF
6.25	0.053	0.022	0.033	0.036	0.033	0.051
12.5	0.097	0.036	0.038	0.066	0.035	0.045
25	0.122	0.066	0.075	0.113	0.085	0.12
50	0.241	0.118	0.123	0.197	0.143	0.175
100	0.471	0.209	0.277	0.348	0.251	0.333

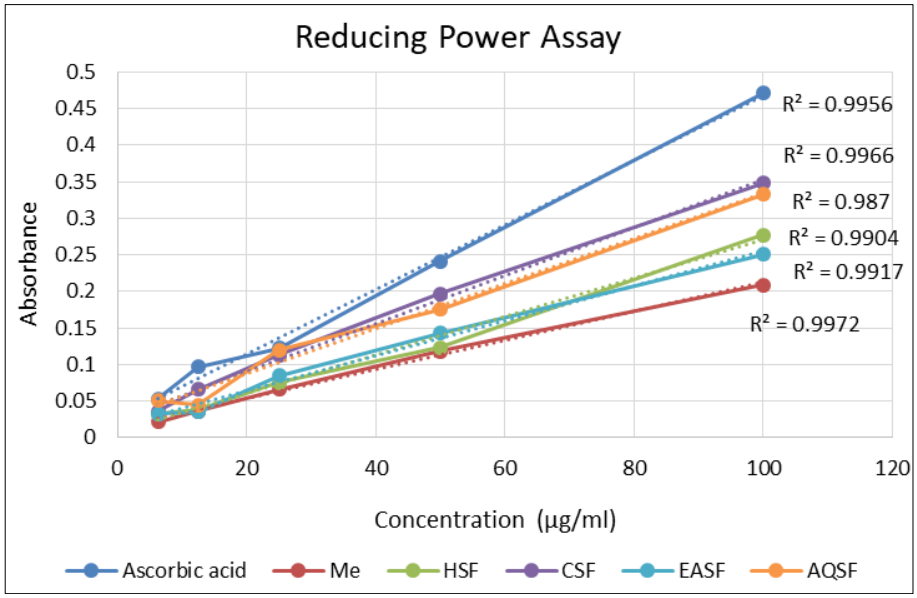

Fig 2: Comparison of reducing power activity of the crude methanolic extract and its different soluble fractions of *C. benghalensis*.

The hydroxyl radical scavenging activity of the methanolic extract and its different soluble fractions (*n*-hexane, chloroform, ethyl acetate and aqueous) was dependent on the dose. Between the methanolic extract and it's soluble fractions, higher hydroxyl radical scavenging activity was shown by the *n*-hexane soluble fraction with IC_{50} value 58.58 ± 1.21 µg/ml. At a concentration of 200 µg/ml, the scavenging activity of the methanolic extract and *n*-hexane, chloroform, ethyl acetate and aqueous soluble fraction was 69.32 ± 0.98, 68.93 ± 0.17, 61.18 ± 0.36, 63.27 ± 28 and 63 ± 0.33 %, respectively, whereas at the same concentration, the standard catechin was 81.79 ± 0.23 % (Figure 3). The hydroxyl radical scavenging activity of the aqueous soluble fraction was closest to that of catechin (standard). The IC_{50} of of the methanolic extract and its *n*-hexane, chloroform, ethyl acetate and aqueous soluble fractions and standard catechin was 71.89 ± 0.52, 58.58 ± 1.21, 166.68 ± 0.89, 104.33 ± 1.28 and 96.55 ± 1.17 µg/ml, respectively, demonstrating that the inhibitory activity of *n*-hexane soluble fraction was higher than standard catechin, IC_{50} 89.61 ± 0.43 µg/ml (Table 4).

Table 4: Hydroxyl radical scavenging activity (IC_{50} in µg/ml) of extractives of roots of C. benghalensis growing in Bangladesh

Test material	IC_{50} (µg/ml)
Me	71.89 ± 1.09
HSF	58.58 ± 1.20
CHSF	166.68 ± 0.54
EASF	104.33 ± 0.05
AQSF	96.55 ± 0.75
Catechin	89.61 ± 0.43

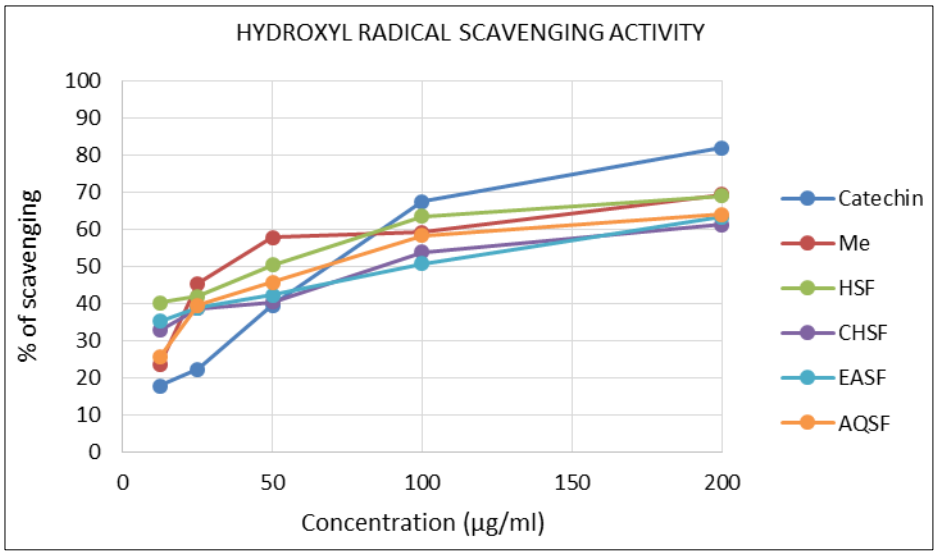

Fig 3: Comparison of reducing power activity of the crude methanolic extract and its different soluble fractions of *C. benghalensis*.

Phosphomolybdenum method was used to assay the total antioxidant capacity of the methanolic extract and its four soluble fractions (HSF, CHSF, EASF and AQSF) and expressed as equivalents of ascorbic acid as shown in Figure 4. According to Figure 4 all the samples showed total antioxidant activity in a concentration dependent. At 100 µg/ml highest absorbance was shown by the HSF (0.743 ± 0.003), followed by the CHSF (0.681 ± 0.002), Me (0.247 ± 0.002), EASF (0.142 ± 0.002) and AQSF (0.110 ± 0.010) and was found to decrease in order of HSF > CHSF > Me > EASF > AQSF.

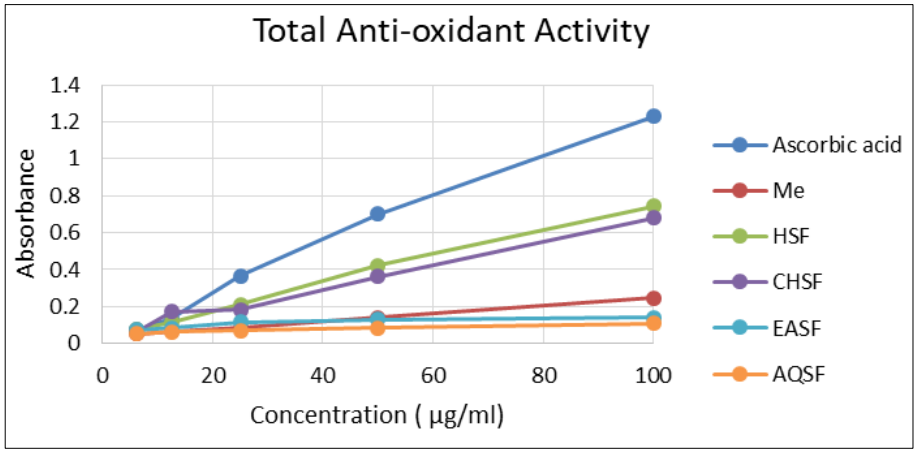

Fig 4: Comparison of total anti-oxidant activity of the crude methanolic extract and its different soluble fractions of *C. benghalensis*

The amount of total phenolic content varied for the methanol extract and different partitionates ranging from approximately 9.5 mg to 37.5 mg of GAE/gm of dried extract. The highest total phenolic content was found from ethyl acetate soluble fraction (37.41 ± 0.002 mg of GAE/gm of dried extract) and the lowest in *n*-hexane soluble fraction (9.57 ± 0.005 mg of GAE/gm of dried extract). All the results were taken three times and mean of the triplicates are given in Table 5. Similar to the total phenolic content, the highest flavonoid content was observed from the ethyl acetate soluble fraction (83.60 ± 0.003 mg of CA/gm of dried extract) and the lowest in *n*-hexane soluble fraction (32.26 ± 0.003 mg of GAE/gm of dried extract).

Table 5: Total phenolic content and flavonoid content of the methanolic extract and its four soluble fractions

	Phenolics[a]	Flavonoids[b]
Me	10.24 ± 0.004	38.93 ± 0.002
HSF	9.57 ± 0.005	32.26 ± 0.003
CHSF	29.66 ± 0.004	56.66 ± 0.002
EASF	37.41 ± 0.002	83.60 ± 0.003
AQSF	16.16 ± 0.004	35.73 ± 0.004

NB: Average of triplicates ± SD, a and b expressed as GAE & CAE, respectively (mg of GA or CA/ gm of dry extract)

4. Discussion

A free radical may be defined as any molecular species capable of independent existence that contains an unpaired electron in an atomic orbital. The presence of an unpaired electron results in certain common properties that are shared by most radicals. Many radicals are unstable and highly reactive. They can either donate an electron to or accept an electron from other molecules, therefore behaving as oxidants or reductants [20]. Free radicals have been claimed to play a key role, affecting human health by causing severe diseases, such as cancer and cardiovascular diseases by cell degeneration. There are increasing suggestions by considerable evidence that these compounds induce oxidative damage to biomolecules (lipids, proteins and nucleic acids) [21]. They also react rapidly with electron acceptors, such as molecular oxygen to become radicals themselves, also referred to as reactive oxygen species (ROS) [22]. Plants are potential sources of natural antioxidants. Fruits and vegetables in the diet have been shown in epidemiological studies to be protective against several chronic diseases associated with aging such as cancer, cardiovascular disease, cataracts, and brain and immune dysfunction [23-25]. These natural protective effects have been attributed to various components such as carotenoids, vitamins C and E, and phenolic and thiol (SH) compounds [26]. Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been used as antioxidants since the beginning of this century. However, restrictions on the use of these compounds are being imposed because of their carcinogenicity and other toxic properties [27]. Thus, the interest in natural antioxidants has increased considerably. These compounds exhibit their antioxidant activity by various mechanisms including chain-breaking by donation of hydrogen atoms or electrons that convert free radicals into more stable species and decomposing lipid peroxides into stable final products [28]. Phenolics are the compounds known as powerful chain breaking antioxidants [29] and phenols are very important plant constituents because of their scavenging ability due to their hydroxyl groups [30]. These compounds have an important role in stabilizing lipid oxidation and are associated with antioxidant activity [31]. The phenolic compounds may contribute directly to antioxidative action. It was suggested that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans, when ingested upto 1gm daily from a diet rich in fruits and vegetables [32]. Flavonoids belong to a group of natural substances with variable phenolic structures and are found in fruit, vegetables, grains, bark, roots, stems, flowers, tea, and wine [33]. More than 4000 varieties of flavonoids have been identified, many of which are responsible for the attractive colors of flowers, fruit, and leaves [34]. The best-described property of almost every group of flavonoids is their capacity to act as antioxidants. The flavones and catechins seem to be the most powerful flavonoids for protecting the body against reactive oxygen species. Body cells and tissues are continuously threatened by the damage caused by free radicals and reactive oxygen species, which are produced during normal oxygen metabolism or are induced by exogenous damage [35, 36].

5. Conclusion

From the thorough study of this experiment it may be concluded that methanol extract along with its *n*-hexane, chloroform, ethyl acetate and aqueous soluble fractions of the roots of Bangladeshi *Coffea benghalensis* B. Heyne ex. Schult has potent antioxidant activities. So, further experiment like compound isolation may gestate what compounds are responsible for these activities. Nevertheless, these studies are preliminary in nature and there are plenty of scopes for further studies like compound isolation by which the discovery of which compounds are responsible for these activities may come to the limelight. Further by carrying out clinical trial to

determine the active compounds responsible for such pharmacological activities may lead to the development of a novel compound for drug discovery. Ultimately, it may lead to the new drug discovery.

6. References

1. Sies H. Oxidative stress: oxidants and antioxidants. Exp Physiol 1997;82(2):291-295.
2. Sies H. Oxidative stress: oxidants and antioxidants. Exp Physiol 1997;82(2):291-295. Journal of Pharmacology 2010;3(9):1-5.
3. Krishnaiah D, Sarbatly R, Nithyanandam R. A review of the antioxidant potential of medicinal plant species. Food Bioprod Process 2011;89:217-33.
4. Szent-Giörgyi A. Lost in the twentieth century. Annu Rev Biochem 1963;36:1-15.
5. Kasote DM, Hegde MV, Katyare SS. Mitochondrial dysfunction in psychiatric and neurological diseases: cause(s), consequence(s), and implications of antioxidant therapy. Biofactors 2013;39:392-06.
6. Sivaranjan VV, Biju SD, Mathew P. Revision of the genus *Psilanthus* Hook.f. (Rubiaceae tribe Coffeeae) in India. Bot Bull of Acade Sinica 1992;33:214-216.
7. Sivaranjan VV, Biju SD, Mathew P. Revision of the genus Psilathus Hook. f. (Rubiaceae tribe Coffeeae) in India. Bot Bull of Acade Sinica 1992;33:214-216.
8. Patay EB, Bencsik T, Papp N. Phytochemical overview and medicinal importance of Coffea species from the past until now. Asi Pac J Tropi Med 2016;9(12):1127-1135.
9. Patay EB, Nemeth TB, Filep R, Vlase L, Papp N. Hsitological and phytochemical studies of Coffea benghalensis B. Heyne Ex Schult., compared with *Coffea arabica* L. Farmacia 2016;64 (1):129.
10. Hasan CM, Huda Q, Lavaud C, Connolly JD, Haque ME. Bengalsol, a new 16-epicafestol derivative from the leaves of *Coffea benghalensis*. Nat Prod Lett 1994;5(1):55.
11. Ghimire K, Rishi Ram Bastakoti RR. Ethnomedical knowledge and healthcare practices among the Tharus of Nawalparasi district in central Nepal. For Eco and Manag 2009;257:2066-2072.
12. VanWagenen AC, Larsen R, Cardellina JH, Ran dazzo D, Lidert ZC, Swithenbank C. J Org Chem 1993;58:335.
13. Braca A, Tommasi ND, Bari LD, Pizza C, Politi M, Morelli I. Antioxidant principles from Bauhinia terapotensis. J Natl Prod 2001;64:892-95.
14. Oyaizu M. Studies on products of browning reaction: Antioxidant activity of products of browning reaction prepared from glucosamine. Jpn J Nutr 1986;44(6):307-315.
15. Halliwell B, Gutteridge JMC, Aruoma OI. Thr deoxyribose method: a simple "Test-Tube" assay for determination of rate constants for reaction of hydroxyl radicals. Anal Biochem 1987;165:215-219.
16. Prieto P, Pineda M, Aguilar M. Sphectrophotometric quanttation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Anal. Biochem 1999;269:337-341.
17. Skerget M, Kotnik P, Hadolin M, Hras A, Simonic M, Knez Z Food Chem 2005;89:191-198.
18. Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem 1999;64:555-559.
19. Maisuthisakul P, Suttajit M, Pongsawatmanit R. Assessment of phenolic content and free radical scavenging capacity of some Thai indigenous plants. Food Chem 2007;100(4):1409-1418.
20. Cheeseman KH, Slater TF. An introduction to free radicals chemistry. Br Med Bull 1993;49:481-493.
21. Shoib AB, Shahid AM. Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of Arisaema jacquemontii Blume. J Taibah Univ Sci 2015;9:449-454.
22. Pincemail JJ. Free radicals and antioxidants in human diseases. In: Favier AE, Cadet J, Kalyanaraman B, Fontecave M, Pierre JL, Editor. Analysis of free radicals, in biological systems. Berlin: Birkhauser Verlag 1995, P83.
23. Ames BN, Shigenaga MK, Hagen TM. Oxidants, antioxidants, and the degenerative diseases of aging. Proceedings of the National Academy of Sciences of the United States of America 1993:90(17):7915-7922.
24. Block G, Patterson B, Subar A. Fruit, vegetables, and cancer prevention: a review of the epidemiological evidence. Nutrition and Cancer 1992;18(1):1-29.
25. Vinson JA, Jang J, Dabbagh YA, Serry MM, Cai S. Plant polyphenols exhibit lipoprotein-bound antioxidant activity using an *in vitro* oxidation model for heart disease. Journal of Agricultural and Food Chemistry 1995;43(11):2798-2799.
26. Paganga G, Miller N, Rice-Evans CA. The polyphenolic content of fruit and vegetables and their antioxidant activities. What does a serving constitute? Free Radical Research 1999;30(2):153-162.
27. Adel FA, Mengjin S, Cunyu L, Wenyi K. Comparative analysis of antioxidant activities of essential oils and extracts of fennel (*Foeniculum vulgare* Mill.) seeds from Egypt and China. Food Sci Human Well 2019;8:67-72.
28. Neelam G, Madhu G, Darshana M, Bhupendra KM. Chemical composition, total phenolic and flavonoid contents, and *in vitro* antimicrobial and antioxidant activities of crude extracts from red chilli seeds (*Capsicum frutescens* L). J Taibah Univ Sci 2016; 10:462-470.
29. Shahidi F, Janitha PK, Wanasundara PD. Phenolic antioxidants. Cri Rev in Food Sci and Nutr 1992;32(1):67-103.
30. Hatano T, Edamatsu R, Hiramatsu M, Mori A, Fujita Y, Yasuhara T *et al*. Effects of interaction of tannins and related polyphenols on superoxide anion radical and DPPH radical. Chem Pharm Bull 1989;37:2016-2021.
31. Yen GC, Duh PD, Tsai CL. Relationship between antioxidant activity and maturity of peanut hulls. J Agric Food Chem 1993;41:67-70.
32. Tanaka M, Kuei CW, Nagashima Y. Application of antioxidative maillrad reaction products from histidine and glucose to sardine products. Nippon Suisan Gakkaishi 1998;54:1409-1414.
33. Middleton EJ. Effect of plant flavonoids on immune and inflammatory cell function. Adv Exp Med Biol 1998;439:175-82.
34. de Groot H, Rauen U. Tissue injury by reactive oxygen species and the protective effects of flavonoids. Fundam Clin Pharmacol 1998;12:249-255.
35. De Groot H. Reactive oxygen species in tissue injury. Hepatogastroenterology 1994;41:328-332.
36. Grace PA. Ischaemia-reperfusion injury. Br J Surg 1994;81:637-647.