

Inspection of Phytochemical Content and *In Vitro* Antioxidant Profile of *Gnaphalium luteoalbum* L.: An Unexplored Phytomedicine

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Abstract: *Background:* Oxidative stress is intensely linked with several pathological manifestations. Searching for medicinal plant with the superior safety profile for the treatment of oxidative stress related disorders are ongoing due to multiple unwanted effects associated with synthetic antioxidants. Therefore the purpose of this study was to examine the phytochemical content, *in vitro* antioxidant potentiality of crude methanol extract (CME), carbon tetrachloride fraction (CTF), petroleum ether fraction (PEF), chloroform fraction (CLF) and ethyl acetate fraction (EAF) of aerial parts of *Gnaphalium luteoalbum* (GL) L.

Methods: The aerial parts of the GL were extracted with methanol followed by fractionation using carbon tetrachloride, petroleum ether, chloroform and ethyl acetate. The phytochemical screening of this plant was performed by using standard methods to evaluate the existence of alkaloids, carbohydrates, phenols, flavonoids, saponins, tannins, terpenoids and fixed oils. Antioxidant potentiality was estimated by, 2,2-diphenyl-1-picrylhydrazyl (DPPH), hydroxyl (OH) and nitric oxide (NO) radical scavenging tests. Total antioxidant capacity (TAC), total phenolic content (TPC) and total flavonoid content (TFC) were also measured.

Results: Phytochemical analysis of the aerial parts of GL confirmed the presence of carbohydrates, phenols, flavonoids and saponins in crude extract and its all fractions. The CME showed the highest scavenging activity (43.28%) with IC₅₀ of 398.49 µg/mL in the DPPH radical scavenging test. The IC₅₀ values of EAF, CME were statistically significant (P < 0.05, P < 0.01) with respect to ascorbic acid (ACA). For OH and NO radical scavenging tests maximum scavenging (48.39%, 69.64%) was also reported for CME compared to CTF, PEF, CLF and EAF. Compared to ACA, in case of OH and NO radical scavenging activities the IC₅₀ values of CME were markedly significant (P < 0.01, P < 0.05). In the TAC test, CME showed the highest antioxidant activity (absorbance, 2.6 nm) related to other fractions. TPC was found to be the highest in the CME (115.96 mg of gallic acid equivalent/g of dried extract) rather than other fractions. The ranking order of CTF, PEF, CLF, EAF and CME for TFC was 48.67 < 55.75 < 65.29 < 71.35 < 82.29 mg quercetin equivalent/g of dried extract.

Conclusion: The existing study suggested that CME of the aerial parts of GL can be used as a natural source of antioxidant which might be effective towards preventing or slowing oxidative stress related disorders.

Keywords: Phytochemical, Antioxidant activity, *Gnaphalium luteoalbum*, Oxidative stress, Synthetic antioxidants.

INTRODUCTION

Oxygen is considered as prime oxidant in metabolic reactions in order to obtain energy from the oxidation of a variety of organic molecules required to sustain life [1]. Oxidative stress is the outcome of the metabolic reactions that use oxygen, which is the disturbance in the equilibrium status of pro-oxidant/anti-oxidant systems in cells owing to excess amount of reactive oxygen/nitrogen species (ROS/RNS) [2]. Disturbances

in the normal redox state of cells can cause toxic effects through the production of free radicals and peroxides that damage all components of the cell, including proteins, lipids and nucleic acid [3]. Free radicals and other reactive species are supposed to play a key role in oxidative stress that is linked with aging and countless chronic diseases such as atherosclerosis, heart disease, arthritis, gastritis, diabetes, cancer, preeclampsia, acquired immune deficiency syndrome (AIDS), inflammation, ischemia and neurodegenerative diseases especially Alzheimer's disease, Huntington disease, Parkinson disease (PD), amyotrophic lateral sclerosis and celiac diseases [4-7].

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Antioxidants are substances that inhibit oxidative damage of other molecules in biological entities. Antioxidants exert its action by slowing or preventing the oxidation process that can damage cells [8]. In case of countering free radical mediated oxidative stress, antioxidants are considered as crucial while the human body has its endogenous antioxidant defenses against oxidative stress [9]. During aging and or in case of certain illness endogenous antioxidants are not sufficient and required exogenous antioxidants [9, 10]. Although, antioxidants are commercially available as dietary supplements but there is no confirming clinical evidence in support of synthetic antioxidants as useful in keeping healthy and preventing disease [11]. Conversely, the safety of synthetic antioxidants (propyl gallate, butylated hydroxyanisole, butylated hydroxytoluene etc.) is dubious and assumed to be oncogenic [12]. In addition, there is a lot of anecdotal proof that antioxidant-rich medicinal plants/foods are highly effective for protection against neurodegenerative diseases, heart disease, cancer, diabetes, muscular degeneration, etc [1, 13].

Phytoconstituents are mainstay for exerting various therapeutic activities and serve as a blueprint for the development of new pharmaceuticals [14]. Worldwide currently, there has been an augmented interest to find antioxidant compounds which are pharmacologically active and have minor or no side effects [15]. Medicinal plants are the greatest source of natural antioxidant. In the developing world, medicinal plants serve as a potential source of primary healthcare needs [16]. The antioxidant activity of medicinal plants are owing to the presence of phytoconstituents such as phenolics (polyphenolics) and flavonoids to prevent the oxidative stress caused by ROS/RNS [15, 16]. Complementary and alternative medicine systems including Ayurveda, Unani Chinese and other afford extensive lead to find therapeutically effective antioxidant compounds from medicinal plants [17].

The plant *Gnaphalium luteoalbum* (GL) L. belongs to the family Asteraceae is known in Bengali as Bara Kamra [18]. This plant is widely distributed all over Bangladesh in fallow lands. It is an annual herb with a height of 15 to 45 cm, stem simple or branched and softly woolly. Leaves are sessile, about 2.5 to 6.3 cm long, spatulate-oblong or linear oblong, woolly on both surfaces [19]. The flowers of this plant are dense, leafless, corymbose clusters of yellow glistening heads [18]. The leaves of this plant are used as an astringent and vulnerary and the tomentum is applied as a counter irritant for gout [20].

A number of studies suggested that this plant contains glucoside resins, tannins, flavonoids, essential oils, phytosterins, etc [21]. Therefore the intention of this study was to examine phytochemical constituents and *in vitro* antioxidant activity of crude methanol extract (CME), carbon tetrachloride fraction (CTF), petroleum ether fraction (PEF), chloroform fraction (CLF) and ethyl acetate fraction (EAF) of aerial parts of GL.

MATERIALS AND METHODS

Chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2-deoxy-2-ribose, EDTA (ethylenediaminetetraacetic acid), sulphuric acid, ammonium molybdate, sodium nitroprusside, sulfanilic acid reagent, thiobarbituric acid (TBA), trichloro acetic acid (TCA), Folin-Ciocalteu (FC) reagent, ascorbic acid (ACA), gallic acid (GA) and quercetin (QR) were bought from Sigma-Aldrich, USA. Unless otherwise specified, remaining chemicals were of analytical grade and obtained from native sources.

Collection and Identification of Plant Materials

The aerial parts of the GL were collected in July, 2016 from Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh and identified by a skilled taxonomist of the same institute. A voucher specimen with accession number has been deposited in Bangladesh National Herbarium, Dhaka, Bangladesh. The accession number of GL was: DACB-39509.

Drying and Grinding of Plant Materials

The aerial parts of the plant were washed properly by using water followed by shade drying and irregular sun drying for 10 days. Then dried aerial parts were ground into coarse powder by using a suitable crushing machine. After that generated powder sample was stored in an airtight glass container until extraction.

Extraction and Fractionation of Plant Materials

Powdered plant materials (i.e., aerial parts) having a weight of 250 g was taken in an amber colored glass bottle and soaked in 900 ml of 98% methanol at 25°C. The bottle with powder sample was sealed and kept for 7 days with unsystematic agitation at 25°C. After that the extract was filtered to obtain filtrate and then concentrated by the help of a rotary evaporator at 50°C temperatures to give crude methanolic extract (CME). The CME was subjected to fraction, by using carbon tetrachloride (CTF), petroleum ether (PEF), chloroform

(CLF) and ethyl acetate (EAF) respectively. Then fractionated solvents were evaporated to produce 2.94 g, 2.72 g, 2.49 g and 2.15 g fractions respectively and kept until antioxidant tests.

Phytochemical Analysis

The phytochemical screening of the crude extract and its all fractions were determined by using the method as described by Sofowora to detect the presence or absence of secondary metabolites such as alkaloids, carbohydrates, phenols, flavonoids, saponins, tannins, terpenoids and fixed oils [22].

Antioxidant Activity

To start antioxidant activity a solution of each obtained plant extract/fractions was made by using 95% methanol at 1 mg/ml concentration.

DPPH Radical Scavenging Assay

In line with the method of Choi *et al.*, with minor modifications DPPH radical scavenging activity of the GL was examined [23]. In this test, 2 ml of sample (extract/fractions) at numerous concentrations (i.e., 25 to 400 µg/ml) was added to 3 ml 0.02 % of methanol solution of DPPH into the test tubes. To complete the reaction the test tubes were incubated for 30 min in dark place. Then the absorbance was measured at 517 nm in contradiction of methanol as blank using UV spectrophotometer. For this test as a standard ascorbic acid (ACA) was used.

The following equation was used to calculate the percent scavenging of the DPPH free radical:

$$\text{DPPH scavenging (\%)} = [1 - (A/A_0)] \times 100$$

where, A symbolizes the absorbance of the sample/standard solution and A_0 symbolizes the absorbance of the control.

Hydroxyl Radical Scavenging Assay

In line with the method of Elizabeth *et al.*, hydroxyl (OH) radical scavenging activity of the GL was examined [24]. In this test, 0.1 ml of sample (extract/fractions) at various concentrations (i.e., 25 to 400 µg/ml) was added to 1 ml of reaction mixture [0.5 ml of 2.8 mM 2-deoxyribose in a 50 mM phosphate buffer (pH 7.4), 0.2 ml of premixed 100 µM ferric chloride and 100 µM EDTA (1:1; v/v), 0.1 ml of 200 mM hydrogen peroxide and 0.1 ml 300 µM ACA] into the test tubes. After an incubation period of 1 h at 37°C,

0.5 ml of the reaction mixture was added to 1 ml 2.8% TCA followed by addition of 1 ml 1% TBA solution and then the reaction mixture was incubated at 90°C for 15 min so that color develop. Then at 25°C test tubes were cooled and the absorbance was measured at 532 nm in contradiction of methanol as blank using UV spectrophotometer. For this test as a standard ACA was used.

The following equation was used to calculate the percent scavenging of the OH free radical:

$$\text{OH radical scavenging (\%)} = [1 - (A/A_0)] \times 100$$

where, A symbolizes the absorbance of the sample/standard solution and A_0 symbolizes the absorbance of the control.

Nitric Oxide Radical Scavenging Assay

In line with the method of Rai *et al.*, nitric oxide (NO) radical scavenging activity of the GL was examined [25]. In this test, 0.5 ml of sample (extract/fractions) at various concentrations (i.e., 25 to 400 µg/ml) was added to 0.5 ml of 10 mM sodium nitroprusside in phosphate buffered-saline into the test tubes. After an incubation period of 150 min at 25°C in the dark, 1 ml of sulfanilic acid reagent (0.33% sulfanilic acid in 20% glacial acetic acid) was added to 0.5 ml of the reaction mixture so that color develop. Then the test tubes were again incubated for 5 min followed by addition of 1 ml of 0.1% naphthyl ethylene diamine dihydrochloride and again incubated for 30 min at 25°C. Then the absorbance was measured at 540 nm in contradiction of methanol as blank using UV spectrophotometer. For this test as a standard ACA was used.

The following equation was used to calculate the percent scavenging of the NO free radical:

$$\text{NO radical scavenging (\%)} = [1 - (A/A_0)] \times 100$$

where, A symbolizes the absorbance of the sample/standard solution and A_0 symbolizes the absorbance of the control.

Total Antioxidant Capacity (TAC)

In line with the method of Prieto *et al.*, with minor modifications TAC of the GL was examined [26]. In this test, 0.5 ml of sample (extract/fractions) at various concentrations (i.e., 100-600 µg/ml) was added to 3 ml of reaction mixture (28 mM sodium phosphate, 0.6 M sulphuric acid and 1% ammonium molybdate) into the test tubes. To complete the reaction the test tubes

were incubated for 15 min at 95°C in a water bath. Then the test tubes were cooled up to 25°C and the absorbance was measured at 695 nm in contradiction of a reagent blank by the help of UV spectrophotometer. For this test as a standard ACA was used.

Total Phenolic Content (TPC)

In line with the method as stated by Singleton and Rossi with minor modifications TPC of the GL was examined [27]. In this test 0.1 ml of sample (extract/fractions) with a concentration of 1000 µg/ml was mixed with 0.75 ml of FC reagent that was previously diluted 1000-fold by using distilled water into the test tubes. Then for 5 min the test tubes were incubated at 22°C followed by the addition of 0.06% sodium carbonate solution and again incubated for 90 min at 22°C to complete the reaction. Then the absorbance was measured at 725 nm in contradiction of a reagent blank by the help of UV spectrophotometer. For this test to estimate TPC gallic acid standard curve was used and results were expressed as mg of gallic acid equivalents (GAE)/g of dried sample.

Total Flavonoid Content (TFC)

In line with the method as stated by Chang *et al.*, TFC of GL was examined [28]. In this test 0.1 ml of sample (extract/fractions) with a concentration of 1000 µg/ml was mixed with 3 ml of methanol, 0.2 ml of 10 % aluminum chloride, 0.2 ml of 1 M potassium acetate and 5.6 ml of distilled water into the test tubes. Then for 25 min the test tubes were incubated at 25°C to complete the reaction and the absorbance was measured at 420 nm in contradiction of a reagent blank by the help of UV spectrophotometer. For this test to

estimate TFC quercetin standard curve was used and results were expressed as mg of quercetin equivalents (QRE)/g of dried sample.

Statistical Analysis

The results were expressed as mean \pm standard deviation (SD) from three diverse observations. For *in vitro* antioxidant tests, student's t test was used to find the significance of standard and sample in case of IC₅₀. The statistical and graphical analysis was performed by using SPSS 14.0 (Chicago, IL, USA) and Microsoft Excel 2010 (Roselle, IL, USA). The value of $P < 0.05$ was considered as significant.

RESULTS

Determination of Phytochemical Constituents

Table 1 showed the phytochemical constituents of the GL extract and fractions based on the intensity of the characteristic color. The results confirmed the presence of alkaloids, carbohydrates, phenols, flavonoids, saponins, tannins and terpenoids in CME. Carbohydrates, phenols, flavonoids and saponins were present in CTF, PEF, CLF and EAF.

Determination of DPPH Radical Scavenging Activity

Figure 1 characterizes the DPPH radical scavenging activity of the GL extract and fractions. The radical scavenging activity of the extract/fractions was in the following order: CTF < PEF < CLF < EAF < CME < ACA. Dose-dependent increase in DPPH radical scavenging activity was reported, i.e., antioxidant activity increased with respect to increased concentration of the extract/fractions. Table 2

Table 1: Phytochemical Constituent of the Aerial Part of GL Extract and Fractions

Phytochemical Constituents	CTF	PEF	CLF	EAF	CME
Alkaloids	—	+	+	+	+
Carbohydrates	+	+	++	+	++
Phenols	+	+	+	+	++
Flavonoids	+	+	++	+	+++
Saponins	+	+	+	++	+
Tannins	+	—	+	—	+
Terpenoids	—	+	+	+	+
Fixed oils	+	—	—	—	—

where based on the intensity of the characteristic color, + = Present in mild amount, ++ = Present in moderate amount, +++ = Present in large amount, — = Not present. CTF = Carbon tetrachloride fraction, PEF = Petroleum ether fraction, CLF = Chloroform fraction, EAF = Ethyl acetate fraction, CME = Crude methanol extract.

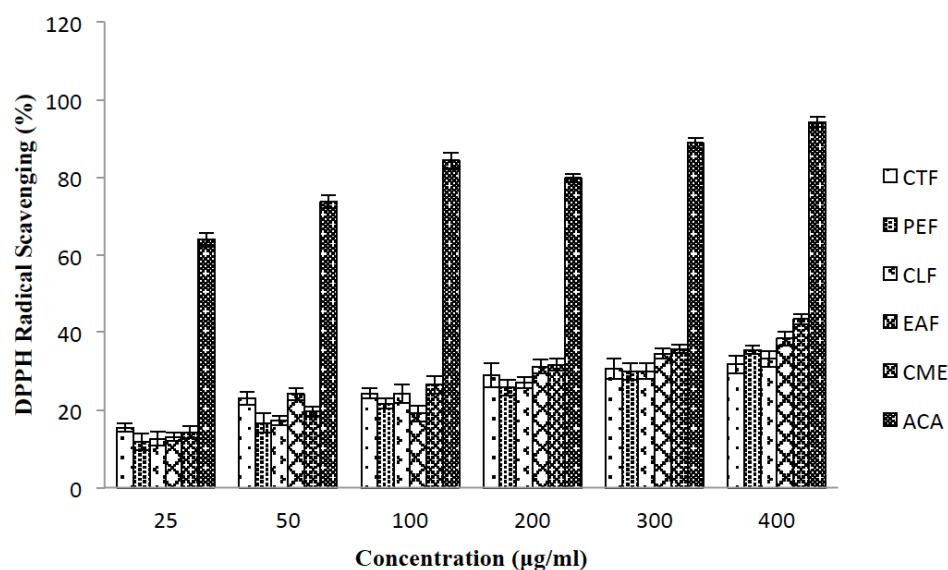


Figure 1: DPPH radical scavenging activity of the aerial part of GL extract and fractions at various concentrations. Values were expressed as mean \pm SD ($n = 3$). CTF = Carbon tetrachloride fraction, PEF = Petroleum ether fraction, CLF = Chloroform fraction, EAF = Ethyl acetate fraction, CME = Crude methanol extract, ACA = Ascorbic acid.

Table 2: IC_{50} Values of the Aerial Part of GL Extract and Fractions for DPPH, OH and NO Radical Scavenging Activity

Sample/Standard	IC_{50} ($\mu\text{g/ml}$)		
	DPPH Radical	OH Radical	NO Radical
CTF	601.33 ± 2.16	478.37 ± 3.28	517.38 ± 2.69
PEF	576.78 ± 3.57	446.56 ± 4.29	486.56 ± 3.45
CLF	523.59 ± 3.13	408.92 ± 2.76	454.73 ± 4.47
EAF	$452.14 \pm 2.88^*$	367.49 ± 4.25	387.49 ± 3.19
CME	$398.49 \pm 4.12^{**}$	$342.23 \pm 3.18^{**}$	$361.53 \pm 4.42^*$
ACA	32.46 ± 3.14	38.91 ± 2.76	47.18 ± 3.79

Values were expressed as mean \pm SD ($n = 3$). CTF = Carbon tetrachloride fraction, PEF = Petroleum ether fraction, CLF = Chloroform fraction, EAF = Ethyl acetate fraction, CME = Crude methanol extract, ACA = Ascorbic acid. * $P < 0.05$, ** $P < 0.01$ significant difference as compared to standard.

represents The IC_{50} values of CTF, PEF, CLF, EAF, CME and ACA in which IC_{50} values of EAF and CME were statistically significant ($P < 0.05$, $P < 0.01$) with respect to ACA.

Determination of OH Radical Scavenging Activity

The OH radical scavenging effect of the GL extract and fractions is given in Figure 2. The radical scavenging activity was in the following order: CTF < PEF < CLF < EAF < CME < ACA. The IC_{50} values of CLF, EF, MNF, CEE and ASC were shown in Table 2. Compared to ACA the IC_{50} value of CME was statistically significant ($P < 0.01$).

Determination of NO Radical Scavenging Activity

In Figure 3, NO radical scavenging activity of the GL extract and fractions is stated in the following order: CTF < PEF < CLF < EAF < CME < ACA. The CME

showed the highest nitric oxide scavenging activity (69.64%) compared to other fractions and the IC_{50} value ($361.53 \pm 4.42 \mu\text{g/ml}$) of this extract was statistically significant ($P < 0.05$) compared to ACA.

Determination of TAC

Figure 4 represents the TAC of the GL extract and fractions in which the antioxidant activity were in the following order: CTF < PEF < CLF < EAF < CME < ACA. The relationship between antioxidant activity and concentration of the extract and fractions was dose-dependent.

Determination of TPC

TPC of the GL extract and fractions was calculated from the standard curve of gallic acid ($y = 0.0158x + 0.0448$; $R^2 = 0.9923$). Among the extract and fraction highest TPC was found in CME compared to the

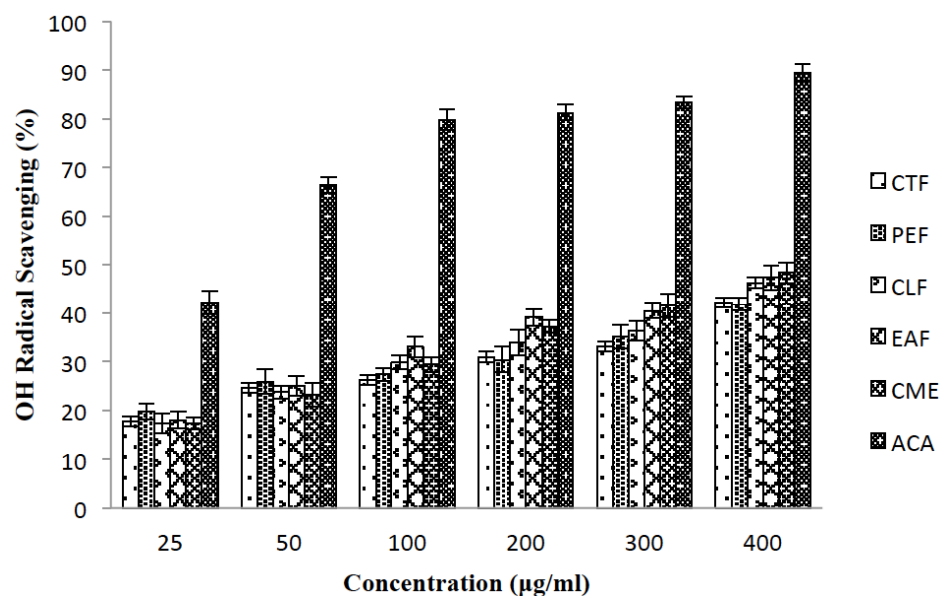


Figure 2: OH radical scavenging activity of the aerial parts of GL extract and fractions at various concentrations. Values were expressed as mean \pm SD ($n = 3$). CTF = Carbon tetrachloride fraction, PEF = Petroleum ether fraction, CLF = Chloroform fraction, EAF = Ethyl acetate fraction, CME = Crude methanol extract, ACA = Ascorbic acid.

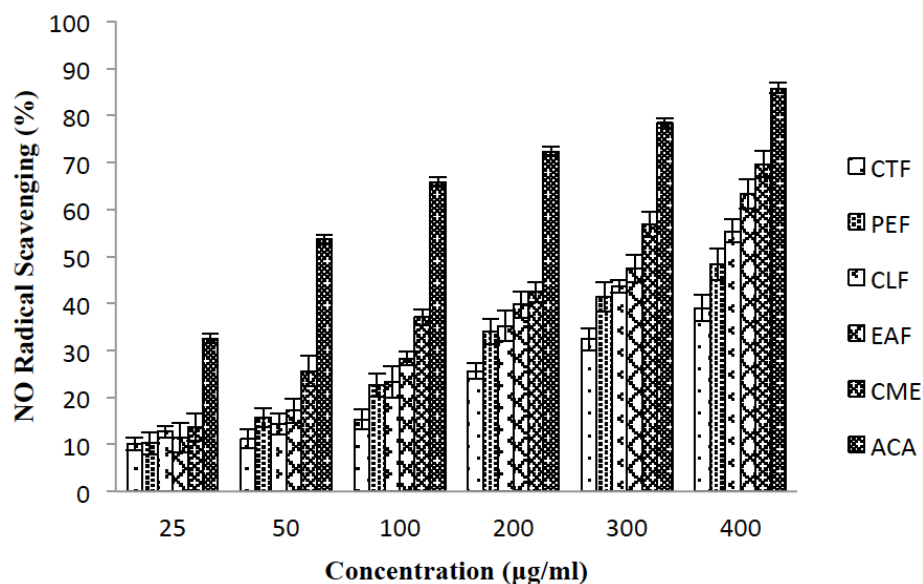


Figure 3: NO radical scavenging activity of the aerial parts of GL extract and fractions at various concentrations. Values were expressed as mean \pm SD ($n = 3$). CTF = Carbon tetrachloride fraction, PEF = Petroleum ether fraction, CLF = Chloroform fraction, EAF = Ethyl acetate fraction, CME = Crude methanol extract, ACA = Ascorbic acid.

remaining fraction given in Figure 5. The following order was founded based on the outcomes of the given figure: CTF < PEF < CLF < EAF < CME.

Determination of TFC

TFC of the GL extract and fractions were calculated from the standard curve of quercetin ($y = 0.0098x + 0.1177$; $R^2 = 0.9908$). Figure 6 represents that the TPC were in the following order: CTF < PEF < CLF < EAF < CME. According to this sequence, CME exhibited the highest flavonoid contents than other fractions.

DISCUSSION

Oxidative stress induced by an increase in free radicals and or decrease in antioxidant defenses is responsible for pathogenesis of various disease states [29]. ROS and RNS play an imperative role in the oxidative stress as specified previously. Recently there has been an upsurge of interest in the therapeutic potential of medicinal plants with antioxidant potency [30]. In the present study, phytochemical content and *in vitro* antioxidant profiling of GL was carried out by using various tests.

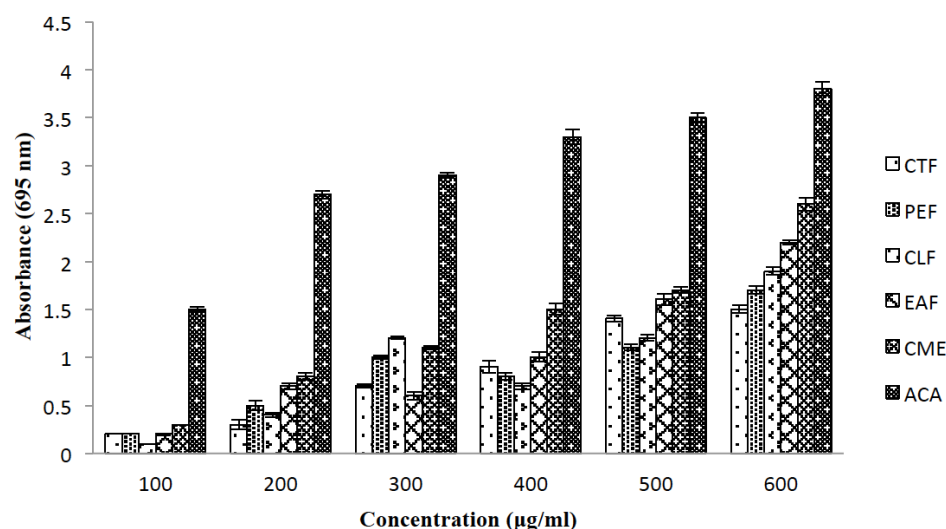


Figure 4: TAC of the aerial parts of GL extract and fractions at various concentrations. Values were expressed as mean \pm SD ($n = 3$). CTF = Carbon tetrachloride fraction, PEF = Petroleum ether fraction, CLF = Chloroform fraction, EAF = Ethyl acetate fraction, CME = Crude methanol extract, ACA = Ascorbic acid.

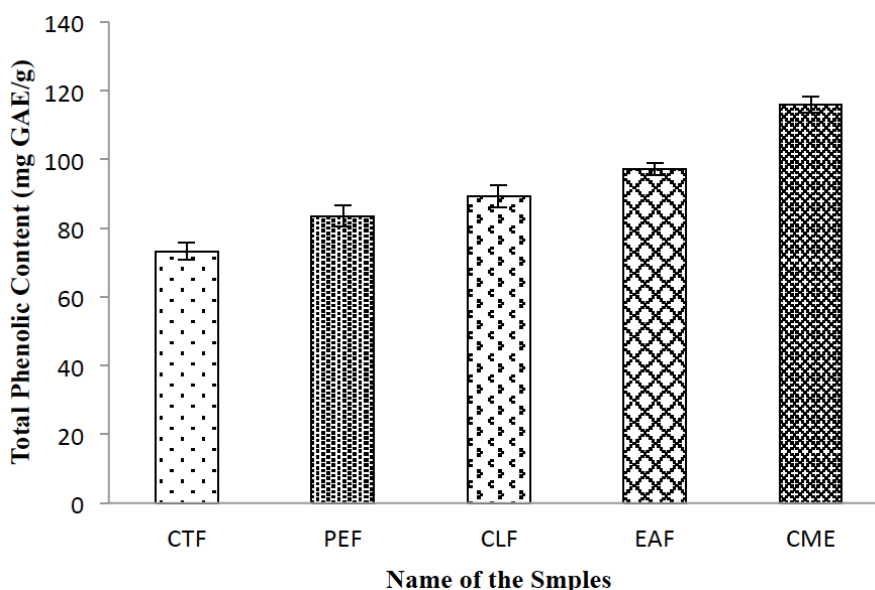


Figure 5: TPC of the aerial parts of GL extract and fractions at various concentrations. Values were expressed as mean \pm SD ($n = 3$). CTF = Carbon tetrachloride fraction, PEF = Petroleum ether fraction, CLF = Chloroform fraction, EAF = Ethyl acetate fraction, CME = Crude methanol extract.

The phytochemical screening of crude extract and its fractions revealed a wide range of phytoconstituents such as alkaloids, carbohydrates, phenols, flavonoids, tannins and terpenoids. Among the phytochemicals, phenols, flavonoids, tannins and terpenoids are accountable for antioxidant activities [31]. Phenolic compounds are strong antioxidants responsible for the protection of oxidative damage to biomolecules (i.e., DNA, lipids and proteins) crucial for numerous diseases [32]. The antioxidant properties of phenols are owing to holding one or more aromatic rings with one or more hydroxyl groups [33]. Plant phenolics include phenolic acids, flavonoids, tannins etc. are the

abundant secondary metabolite have been connected with multiple biological roles such as free radical scavenger, anti-inflammatory, anticancer, hypoglycaemic, hypocholesterolemic antibacterial, antifungal, antiviral activity, antiparasitic etc. activities [34].

The electron gifting capability of the medicinal plant is most widely determined by DPPH radical scavenging test owing to its reliability [35]. In this test, purple-colored solution of DPPH radical by accepting electrons converted to discolor DPPH-H. In fact the degree of color change is related to the concentration and effectiveness of the antioxidants. The degree of

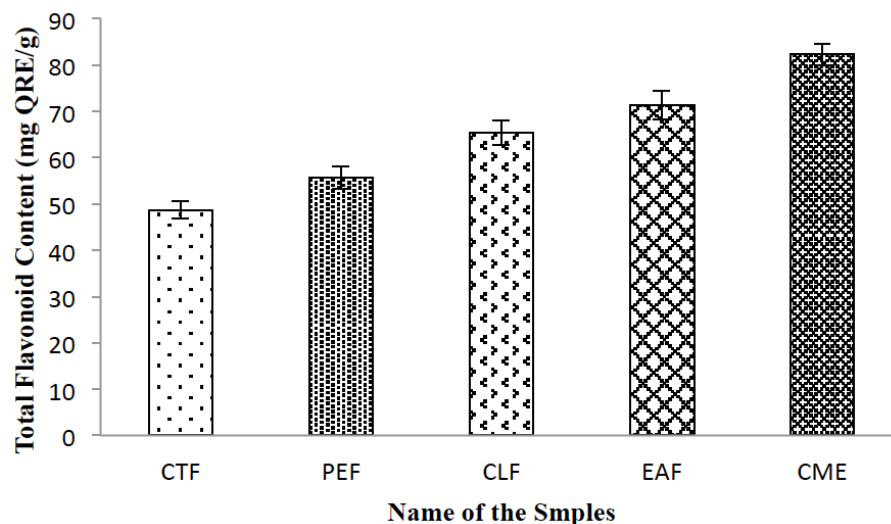


Figure 6: TFC of the aerial parts of GL extract and fractions at various concentrations. Values were expressed as mean \pm SD ($n = 3$). CTF: Carbon tetrachloride fraction, PEF: Petroleum ether fraction, CLF = Chloroform fraction, EAF = Ethyl acetate fraction, CME = Crude methanol extract.

discoloration with respect to decrease in the absorbance of the reaction mixture indicates free radical scavenging action [36]. In the study among the extract and fractions tested, CME exhibited higher percentage of scavenging. The study suggests that the plant extract that contains flavonoid and related polyphenols are proficient for donating hydrogen to a free radical to prevent the disease related to free radical mediated oxidative stress [37].

The OH radical is highly reactive short-lived (approximately 10^{-9} sec) ROS in the biological system that can damage almost all types of macromolecules: carbohydrates, nucleic acids, lipids and amino acids [38]. OH radical shows a significant role in the pathogenesis of biological systems and contributes to neurodegeneration, mutagenesis and carcinogenesis [39]. The source of this radical is mainly the decomposition of hydroperoxides, however byproduct of immune action may also contribute [40]. In this test the formation of low intensity of the red color solution indicates OH radical scavenging power connected to antioxidant capacity. Herein CME showed the highest percentage of scavenging activity compared to remaining fractions. The OH radical scavenging activity of plant extract is responsible for reduction of lipid peroxidation which is considered as leading causative factor for disease [41].

NO is a free radical generated endogenously in several types of cells [42]. It serves as an important biological messenger (i.e., cellular signaling molecule) involved in many physiological plus pathological processes [43]. A high concentration of NO displays

neurotoxicity and can induce apoptotic cell death in different types of neuronal cells. It has been suggested that NO-facilitated neuronal injury is involved in several neuronal disorders such as PD [44, 45]. The results of this study showed the CME has substantial NO radical scavenging activity, compared to remaining fractions in a dose-dependent manner. The radical scavenging antioxidant activity of plant extract mainly depends on the presence of phytoconstituents especially total polyphenols content [46].

The phosphomolybdate test has usually been used to assess the TAC of medicinal plants [47]. This test is based on reduction of molybdenum (Mo) (VI) to the Mo (V) [47, 48]. The formation of green phosphate color Mo (V) compounds from Mo (VI) with a maximum absorption at 695 nm indicates the antioxidant capacity of the plant extract [49]. The existing study confirmed that CME showed the highest antioxidant capacity with respect to remaining fractions. The study recommends that the antioxidant activity of plant extract is noticeable for the presence of bio-active constituents generally polyphenols, tocopherol, carotenoids and ascorbic acid [50].

Phenolic compounds abundant in medicinal plants contribute significantly to the antioxidant properties [51]. The OH groups and conjugated ring structures of the phenolic compounds is responsible for antioxidant effect by scavenging or stabilizing free radicals through hydrogenation or complexing with oxidizing species [52]. Among the phenolic compounds, flavonoids are chief hydroxylated phenolic compounds [53]. A number of studies reported various pharmacological activities of

flavonoidic derivatives, including antioxidant, anti-inflammatory, analgesic, antibacterial, antiviral, anticancer, anti-allergic, antiobesity, antidiabetic and nootropic actions [54]. In the scavenging of various free radicals, flavonoids are highly effective by their redox potential [55]. There has been increasing interest in the research on flavonoids obtained from plants since pharmacological properties of flavonoids are directly linked with their antioxidant potentiality [55, 56]. The results of this study endorsed that polyphenolic constituents (i.e., phenolic acids and flavonoids) may be the foremost contributing agents for the antioxidant activity, in this study highest phenolic and flavonoid contents were reported for CME. An earlier study also exposed almost similar findings [57].

This study revealed a potent *in vitro* antioxidant activity of the aerial parts of GL, which may be an effective source of natural antioxidant.

CONCLUSION

This study evaluated that the CME of the aerial parts of GL exhibited significant antioxidant activity and contained substantial amount of phenols and flavonoids with respect to remaining fractions. The existing study indorsed the presence of lead compounds in GL. So aerial parts of GL can be a good source of natural antioxidant alternatives, yet further studies will be required to isolate the bioactive compound(s).

ABBREVIATIONS

CTF = Carbon tetrachloride fraction

PEF = Petroleum ether fraction

CLF = Chloroform fraction

EAF = Ethyl acetate fraction

CME = Crude methanol extract

ACA = Ascorbic acid

GL = *Gnaphalium luteoalbum*

DPPH = 2,2-Diphenyl-1-picrylhydrazyl

OH = Hydroxyl

NO = Nitric oxide

TAC = Total antioxidant capacity

TFC = Total phenolic content

TFC = Total flavonoid content

ACA = Ascorbic acid

ROS = Reactive oxygen species

RNS = Reactive nitrogen species

AIDS = Acquired immune deficiency syndrome

PD = Parkinson disease

EDTA = Ethylenediaminetetraacetic acid

TBA = Thiobarbituric acid

TCA = Trichloro acetic acid

FC = Folin-Ciocalteu

GAE = Gallic acid equivalents

QRE = Quercetin equivalents

Mo = Molybdenum

SD = Standard deviation

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between all authors. Author MSU designed the study, wrote the protocol, managed the analyses of the study and prepared the draft of the manuscript. Authors MSU, GMSU and MMI carried out the tests. Author MMB performed literature review. Author OHC performed the statistical analysis. Authors YB and MMAD reviewed the scientific contents of the manuscript. All the authors read and approved the final manuscript.

ACKNOWLEDGEMENTS

The authors wish to thank the anonymous reviewer(s)/editor(s) of this article for their constructive reviews. The authors are also grateful to the Department of Pharmacy, Southeast University, Dhaka, Bangladesh.

COMPETING INTERESTS

The authors proclaim that they have no competing interests.

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