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In vitro Antioxidant Study and Determination of Flavonoids, Flavonols, Total Phenolic and Proanthocyanidins Content of *Grewia abutilifolia* Leaf Extracts

Étude in vitro sur les antioxydants et détermination des teneurs en flavonoïdes, en flavonols, en proanthocyanidines et de la teneur totale en composés phénoliques d'extraits de feuilles de *Grewia abutilifolia*

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Abstract *Grewia abutilifolia* (Tiliaceae) tree is known for its uses in traditional medicine due to its various degrees of ethno-pharmacological activities. In this study, *Grewia abutilifolia* was evaluated for its in vitro antioxidant activities; further, flavonoids, flavonols, total phenolic content and proanthocyanidins content were determined to justify its uses against various oxidative stresses. We evaluated antioxidant activity by determining total antioxidant, Fe^{3+} reducing power, DPPH (2,2-diphenyl-1-picrylhydrazyl) and hydroxyl radical scavenging, and lipid peroxidation inhibition activity. Aqueous fraction (AQF) contained highest total phenolic content (238.56 mg of gallic acid equivalent/g). Crude methanolic extract (CHE) contained maximum flavonoid (211.54 mg) and flavonols (380.25 mg) content equivalent to quercetin which showed significant reducing activity. Proanthocyanidins content was highest in petroleum ether fraction (PEF), 45.89 mg of catechin equivalent per gram. PEF showed maximum activity in DPPH scavenging assay (IC_{50} = 3.82 ± 0.055 µg/ml) and in hydroxyl radical scavenging assay (IC_{50}, concentration required to inhibit the process by half, of 6.45 ± 1.297 µg/ml). Lipid peroxidation inhibition activity was also performed in the test chloroform fraction (CLF), which showed the highest inhibition (IC_{50} = 15.62 ± 1.31 µg/ml). These results suggested its potentiality against various diseases related to oxidative stress.

Keywords Antioxidant · Oxidation · Reducing · Inhibition · *Grewia abutilifolia*

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Résumé Le *Grewia abutilifolia* (Tiliaceae) est un arbre connu pour être utilisé dans la médecine traditionnelle avec divers degrés d'activité ethnopharmacologique. Dans cette étude, le *Grewia abutilifolia* a fait l'objet d'analyses visant à évaluer ses activités antioxydantes in vitro et à déterminer ses teneurs en flavonoïdes, en flavonols, en proanthocyanidines et sa teneur totale en composés phénoliques pour justifier son utilisation contre divers types de stress oxydatif. Pour déterminer les phytoconstituants potentiels, nous avons étudié les teneurs totales en composés phénoliques, en flavonoïdes, en flavonols et en proanthocyanidines. Nous avons évalué l'activité antioxydante en déterminant l'activité antioxydante totale, le pouvoir réducteur des ions Fe^{3+}, en appliquant la méthode du piégeage du DPPH et de radicaux hydroxyles et en déterminant l'activité d'inhibition de la peroxydation lipidique. La fraction aqueuse (AQF) contenait la teneur totale en composés phénoliques la plus élevée (238,56 mg d'équivalent acide gallique/gm). L'extrait brut méthanolique (CHE) contenait une teneur maximale en flavonoïdes (211,54 mg) et en flavonols (380,25 mg) équivalente à la quercétine, ce qui représentait une activité réductrice significative. La teneur en proanthocyanidines était la plus élevée dans la fraction d'éther de pétrole (PEF), soit 45,89 mg d'équivalent catéchine par gramme. La PEF affichait une activité maximale dans le test de la méthode de piégeage du DPPH (IC_{50} = 3,82 ± 0,055 µg/ml) et dans le test de la méthode de piégeage des radicaux hydroxyles (IC_{50} de 6,45 ± 1,297 µg/ml). L'activité d'inhibition de la peroxydation lipidique a également été observée dans la fraction de chloroforme du test (CLF) présentant l'inhibition la plus élevée (IC_{50} = 15,62 ± 1,31 µg/ml). Ces résultats ont indiqué son potentiel contre diverses maladies liées au stress oxydatif.

Mots clés Antioxydant · Oxydation · Réduction · Inhibition · *Grewia abutilifolia*

Introduction

Research on free radicals confirmed that food rich in antioxidants play an essential role in the prevention of cardiovascular diseases, cancers, and neurodegenerative diseases. External antioxidants, like antioxidants extracted from plants, can be administered in order to combat free radicals. Therefore, much attention has been focused on the use of natural antioxidants to protect against oxidative stress. Oxidative stress generally occurs due to the imbalance between antioxidants and prooxidants; it can be the aftermath of neurological dysfunctions. In regular human metabolism, different free radicals like hydroxyl, singlet oxygen, nitric oxide, hydrogen peroxide, and superoxide radicals, are continuously generated [1,2]. Especially in living organisms, they play vital roles in cell signaling to inactivate relevant enzymes, which consequently damage important cellular components. Antioxidants have been proved to resist the destruction of beta-cells [3] as well as other cells and inhibit oxidation processes in the human body [4]. Therefore, antioxidants are vital inhibitors of lipid peroxidation and act against oxidative damage or the stress on living cells by natural defense mechanisms [5]. As a defensive mechanism to inhibit prooxidants, antioxidants are used as a protective entity so that reactive oxygen species (ROS) levels are maintained under the cytotoxic threshold by counteracting the imbalance of cell redox homeostasis [6]. Antioxidant defenses are present in various materials that originate naturally from plant sources such as retinol, ascorbic acids, and different scavengers [7,8]. Antioxidants are those compounds which can quench reactive oxygen species without producing any free radicals [9].

Tiliaceae is a family which is classified under genus *Grewia*. Some plants of this genus have been found to be medicinally active; of these medicinally active plants, about 40 species are found only in India [10]. Some are used as folk medicine to treat diarrhea, malaria, typhoid fever, dysentery, cough, small pox, irritable condition of bladder and intestine, rheumatism, and eczema [11]. Various species of this genus have already been reported to present relevant bioactivity. This includes antibacterial [12], antimalarial [13], and antianemic [14] effect. *Grewia abutilifolia* roots are used to treat abscesses; although a high concentration of the stem bark may cause liver injury if ingested through diet. In some tribal regions in India, the plant's leaf paste is used locally for cooling purpose [15]; it is also used to treat diarrhea and dysentery [16]. In Rajasthan, India, its root decoction are being used to treat bone fracture of human or animal [17]. Furthermore, seed extract and seed oil showed antifertility activity in some cases [18]. In recent studies, they have also been found to exhibit some cholinesterase inhibitory activity [19]. However, no study has yet been undertaken to prove that it works against oxidative stress. It was our main objective to find out if *Grewia abutilifolia* had any possible treatment potential by assessing the activities of its leaf extracts.

Materials and methods

Collection of plant

After collection from hill track area of Comilla, Bangladesh, this plant was verified and identified by an expert taxonomist. Sample of the plant was also registered and kept at the Mirpur National Herbarium, Bangladesh for future preservation.

Preparation of extract

Collected leaves of the plants were properly washed and dried under sun to make a powder using a grinding machine. Dried powders of leaves (600 g) were than soaked in 3 liters of methanol in an amber colored bottle for 10 days. After filtering it with Whatman No.1 filter papers, the plant extract was then concentrated using a rotary evaporator to obtain the expected crude extract. The obtained 60 g crude methanol extract was then partitioned by solvent–solvent extraction according to the modified Kupchan method [20] by using petroleum ether, chloroform, and finally water to obtain the various fractions

Determination of phytoconstituents

Determination of total phenolics

The method followed to determine the total phenolic content of *Grewia abutilifolia* was that recorded by Singleton et al. [21] with slight modifications. The reagent used was Folin−Ciocalteu, well-known for total phenolic content determination. First, the standard solution at different concentrations was added to 2.5 ml of Folin–Ciocalteu reagent solution and 2.5 ml of sodium carbonate (7.5%) solution and mixed well for reaction. Exactly the same procedure was followed for 0.5 ml of plant extract. Then the test tubes containing reaction mixture were incubated for 25 min at 25 °C to complete the reaction and the absorbance of the solution was measured at 760 nm. Gallic acid was used as the standard to prepare the standard curve ($Y = 0.007x + 0.124$, $R^2 = 0.994$) against which the total content of phenolic compounds in our studied plant extracts was compared.

Determination of total flavonoids

The aluminum chloride colorimetric method was used to determine the total flavonoids content by using quercetin as a standard [22]. In this method, 1.0 ml of individual fraction of plant extracts and different concentrations of standard

were mixed with 3 ml of methanol, 0.2 ml of 10% aluminum chloride, 0.2 ml of 1M potassium acetate and 5.6 ml of distilled water respectively, and mixed well. Then it was incubated for 25 minutes. After the incubation, absorbance was measured at 420 nm. Then, a standard curve was made ($Y = 0.013x + 0.093$, $R^2 = 0.993$) to compare the result with dried extractives as a quercetin gram equivalent (QE).

Determination of total flavonols

The method described by Kumaran and Karunakaran et al. [23] was utilized to assay the total flavonols contents of the plant extracts. In this study, we made a solution composed of sample standard (2 ml), 2% aluminum chloride dissolved in ethanol (2 ml) and sodium acetate (3 ml) at a concentration of 50 g/l. The solution was incubated at 20 °C for 2.5 h before measuring the absorbance at 440 nm wavelengths. Total flavonols contents were compared with quercetin standard, equivalent per gram of dried extracts.

Determination of total proanthocyanidins

The procedure described by Sun et al. [24] were used to calculate the total amount of proanthocyanidin in *Grewia abutilifolia*. The solution was prepared by mixing 0.1 mg/ml standard or plant extracts, 4% vanillin–methanol mixture, and hydrochloric acid (0.5 ml, 3 ml and 1.5 ml respectively). After mixing, the solution was left to stand for 15 min before measuring the absorbance at 500 nm wavelength. Then, the total proanthocyanidins contents were compared with catechin standard equivalent per gram of dried extracts.

Antioxidant ability assay

Determination of total antioxidant capacity

The method as described by Prieto et al. [25] with some modifications was used to determine total antioxidant capacity. 0.5 ml of extract of different fractions and standard ascorbic acid at various concentrations were mixed with solution containing 1% ammonium molybdate, 0.6 M sulfuric acid and 28 mM sodium phosphate for reaction. It was then incubated for 15 minutes maintaining 90 °C temperature to start the reaction. Then the mixture was cooled to room temperature and absorbance was observed at 695 nm wavelength.

Reducing power capacity assessment

The reducing power was assessed by following the method described by Oyaizu [26]. At first, 2.5 ml potassium buffer was made with concentration of 0.2 M and pH 6.6; then it was mixed with 2.5 ml 1% potassium ferricyanide solution. This mixture was used to react with different concentrations of 1.0 ml plant extracts and standard solution. To perform the reaction, the mixture solution was incubated at 50 °C for 30 minutes and 2.5 ml of trichloro acetic acid (10%) solution was added into the mixture for further reaction. 2.5 ml supernatant solution was withdrawn from the mixture after it was centrifuged at 3 000 g for 10 min. Then, the supernatant was mixed with 2.5 ml of distilled water and 0.5 ml of $FeCl_3$ (0.1%) solution. The absorbance of the solution was measured at 700 nm. We used ascorbic acid as a standard in this test.

Determination of DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity

A method established by Choi et al. [27] was used to determine the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity. 3 ml of methanol solution of DPPH was added into a test tube with 2 ml of plant extract dissolved in methanol; the test tube was incubated at room temperature for 30 minutes in a dark place to complete the reaction. Then the absorbance of the solution was measured at 517 nm using a spectrophotometer against a blank solution.

Determination of hydroxyl radical scavenging assay

Hydroxyl radical scavenging activity of *Grewia abutilifolia* was determined using the method described by Kunchandy et al. [28]. In this study, test samples/standard (6.25–100 μg/ml) were incubated for 1 h at 37 °C; before incubation, 1 ml of reaction mixture was added to the test tube and the reaction mixture was made with ascorbic acid (100 μM), 2-deoxy-D-ribose (2.8 mM), KH_2PO_4–KOH buffer (20 mM, pH 7.4), $FeCl_3$ (100 μM), EDTA (100 μM), and H_2O_2 (1.0 mM). After incubation, 0.5 ml of the reaction mixture was added to 1 ml of 2.8% trichloroacetic acid (TCA). The mixture was again incubated at 90 °C for 15 min after adding 1 ml of 1% aqueous tetra-butyl alcohol (TBA) to develop the color. After cooling, the absorbance was measured at 532 nm against a blank solution. Catechin was used as standard in this test.

Assessment of lipid peroxidation inhibition activity

A modified method of that used by Liu et al. [29] was used to assess the lipid peroxidation inhibition activity. To conduct this study, adult Long Evan rats (net weight 150 g) were anesthetized with sodium phenobarbitone to collect murine brain. The rat brain was processed in a homogenizer with phosphate buffer to produce 1: 10 homogenate; the phosphate buffer concentration and pH was 50 mM and 7.4 respectively. A supernatant was collected by centrifuging for 20 minutes (10,000 rpm, 4 °C) and subjected to testing for lipid peroxidation inhibition activity. Lipid peroxidation inhibition capacity of various fractions of plant extracts were

assessed for different extracts or standard sample for various concentrations by inducing lipid peroxidation with hydrogen peroxide (10 μM). Afterward, 0.5 ml of brain homogenate and 1 ml of KCl (0.15 M) were added with different extracts or standard sample of various concentrations. 100 μl ferric chloride (0.2 nM) was added and incubated with the mixture for 30 min (maintaining temperature at 37 °C). Addition of 2 ml of 0.25 N HCl, 15% TCA, 0.5% butylated hydroxytoluene (BHT), and 0.38% TBA solution stopped the reaction. During lipid peroxidation, low molecular weight end products, generally malondialdehyde (MDA), are formed by oxidation of polyunsaturated fatty acids that may react with two molecules of TBA to give a pinkish red chromogen [30]. The absorbance of pinkish-red color of MDA−TBA complex was measured at 532 nm. (+)-Catechin was used as standard for comparison.

Statistical analysis

In our current study, different percentages of inhibition or scavenging activity were calculated using the following formula,

Percentage of inhibition or scavenging = $\{(A_C - A_{ES})/A_C\} \times 100$ %

where,

A_C = Observed absorbance for control (without sample) and

A_{ES} = Observed absorbance for extract or standard (with sample).

All IC_{50} values were calculated and all results were expressed as mean ± standard deviation (SD) from three independent experiments, where Microsoft Excel 2007 was used as the analytical tool and for graphical representation.

Results

Determination of phytoconstituents

Total phenolic content (TPC)

TPC for different partitionates were measured and calculated by the method involving Folin−Ciocalteu reagent and using gallic acid as standard. It was expressed as equivalent of gallic acid in mg per dried partitionates. TPC of the aqueous fraction was found to be higher than other fractions with a value of 238.56 ± 0.38 mg of GAE/g of dried extractives as shown in table 1.

Total flavonoids (TF)

TF content of extracts was determined by colorimetric method using $AlCl_3$. The total flavonoids of our observed fractions were measured by comparing with gram equivalent of quercetin (standard). Results were denoted as QE/g, which means quercetin equivalent with per gram of dried sample extracts. 211.54 ± 0.155 mg of QE/g flavonoids was observed for crude methanolic extract which was greater than other fractions as shown in table 1.

Total flavonols

This was determined using colorimetric method of aluminum chloride. The total flavonols of our observed fractions were measured by comparing with gram equivalent of quercetin (standard) as denoted by QE/g. Crude methanol extractive contained highest flavonols, 380.25 ± 2.90 mg of QE/g of sample dried extract, which is graphically shown in table 1.

Total proanthocyanidins

Proanthocyanidins content for various fractions of plant extracts were measured with comparison against catechin standard. Catechin equivalent of per gram of dried extracts was used to indicate the prevalence of proanthocyanidins content, which was denoted as CAT/g. The highest proanthocyanidins content was found in PEF, which had 45.89 ± 1.05 mg of CAT Eq/g of dried extract as shown in table 1.

Antioxidant activity

Total antioxidant capacity

Total antioxidant capacity was assessed using the phosphorous−molybdenum method. In this study, PEF gave

Table 1 Total phenolic, flavonoids, flavonols, and proanthocyanidins content of plant extracts

Name of fractions	Total phenolic content (GAE/g of dried sample)	Total flavonoids content (QE/g of dried sample)	Total flavonols content (QE/g of dried sample)	Total proanthocyanidins content (CAT/g of dried sample
CME	210.97 ± 0.47	211.54 ± 0.155	380.25 ± 2.90	6.94 ± 2.10
PEF	198.82 ± 0.54	198.61 ± 0.150	304.44 ± 2.39	45.89 ± 1.05
CLF	49.79 ± 0.23	157.23 ± 0.155	194.92 ± 3.34	16.07 ± 1.60
AQF	238.56 ± 0.38	169.54 ± 0.155	241.90 ± 2.51	22.73 ± 2.10

Values are mean of triplicate experiments and represented as mean ± STD

significant antioxidant activity when compared with catechin. Absorbance of PEF and standard catechin were observed as 0.073 and 0.072 respectively at 7.8125 µg/ml concentration. From figure 1, we can see that the absorbance of those samples were 1.738 and 3.457 respectively.

Reducing power capacity

A compound which has reducing capacity can easily cleave continuous free radical chains by donating hydrogen atoms. This is why the capacity to reduce free radical is an important factor for antioxidant activity [5]. At concentration 7.8125−250 µg/ml, observed absorbance for chloroform (151−0.965) and ascorbic acid (0.167−3.267) are as shown graphically in figure 2.

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity

The model of scavenging the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of a compound. DPPH antioxidant assay is based on the ability of 2,2-diphenyl-1-picrylhydrazyl (DPPH), to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron, which is responsible for the absorbance at 517 nm and also for a visible deep purple color. DPPH is decolorized when it accepts an electron from the antioxidant compound. This decolorization is measured to calculate the scavenging rate expressed as percentage. In figure 3, we have shown the scavenging rates of various fractions of the plants which were highly significant and slightly less than that of BHT. The IC_{50} of BHT, CME, PEF, CLF, and AQF were 2.30 ± 0.002 µg/ml, 3.75 ± 0.079 µg/ml, 3.82 ± 0.055 µg/ml, 3.63 ± 0.024 µg/ml, and 3.72 ± 0.075 µg/ml respectively.

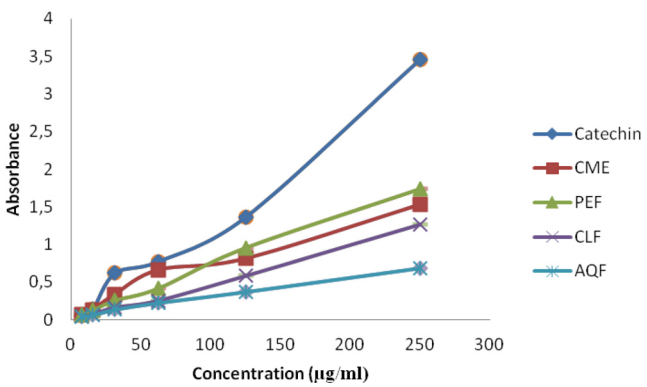

Fig. 1 Total antioxidant activity of different fractions of *Grewia abutilifolia* and standard (catechin)

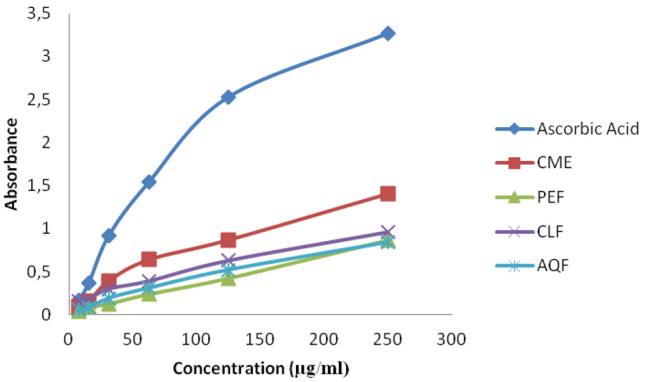

Fig. 2 Reducing power capacities of different fractions of *Grewia abutilifolia* and standard (ascorbic acid)

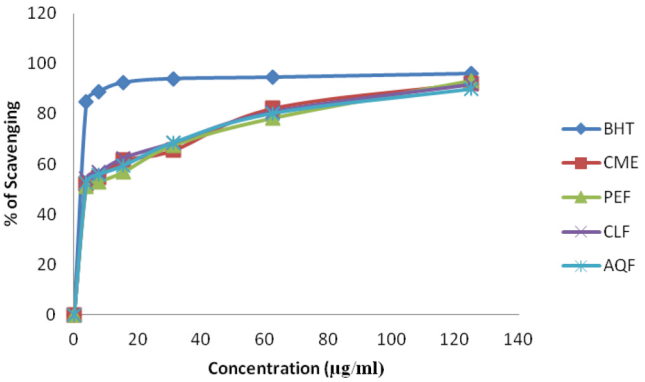

Fig. 3 DPPH radical scavenging activity of different fractions of *Grewia abutilifolia* and standard (BHT)

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity of crude methanol extract and different fractions of *Grewia abutilifolia* are shown in figure 4. Among the fractions of crude methanol extract, PEF showed the most potent activity with IC_{50} value of 6.45 ± 1.297 µg/ml which is lower than that of (+)-catechin (standard) with IC_{50} value of 28.14 ± 0.79 µg/ml. On the other hand, AQF and CLF showed free radical scavenging activity with IC_{50} value of 30.79 ± 1.500 µg/ml and 9.27 ± 0.968 µg/ml respectively as shown in table 2.

Lipid peroxidation inhibition activity

In the lipid peroxidation inhibition assay, the activity of extracts against non-enzymatic lipid peroxidation in rat brain homogenate was evaluated. Addition of Fe^{2+}-ascorbate to the brain homogenate caused an increase in lipid peroxidation which can be quantitatively measured from the change in absorbance at 532 nm and % of inhibition activity of different concentrations as shown in figure 5. Standard catechin (IC_{50} value 6.49 ± 1.001 µg/ml) and

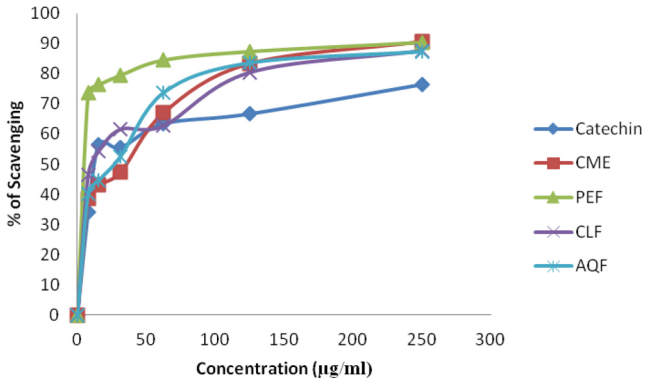

Fig. 4 Hydroxyl radical scavenging activity of different fractions of *Grewia abutilifolia* and standard (catechin)

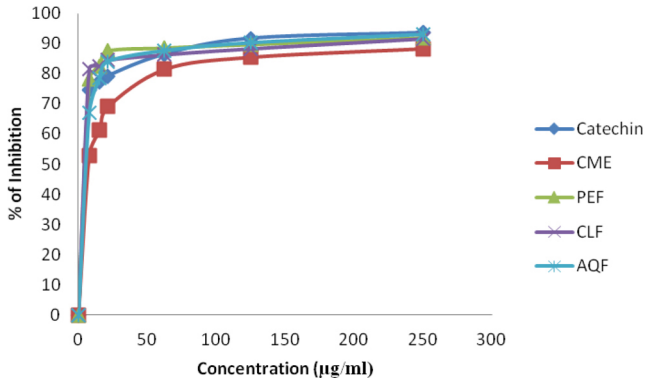

Fig. 5 Lipid peroxidation inhibition activity of different fractions of *Grewia abutilifolia* and standard (catechin)

Table 2 IC_{50} values obtained in the radical scavenging and enzyme inhibitory activity assays

Name of fractions	IC_{50} (µg/ml)		
	DPPH scavenging	Hydroxyl radical scavenging	Inhibition of lipid-peroxidation
BHT	2.30 ± 0.02	–	–
Catechin	–	28.14 ± 0.79	6.49 ± 1.00
Donepezil	–	–	–
Galanthamine	–	–	–
CME	3.75 ± 0.08	32.39 ± 1.08	185.53 ± 3.04
PEF	3.82 ± 0.06	6.45 ± 1.30	117.09 ± 2.01
CLF	3.63 ± 0.02	9.27 ± 0.97	15.62 ± 1.31
AQF	3.72 ± 0.08	30.79 ± 1.50	66.61 ± 1.67

Values are mean of triplicate experiments and represented as mean ± STD

various fractions of crude methanol extract such as petroleum ether fraction (PEF), chloroform fraction (CLF), and aqueous fraction (AQF) showed inhibition of lipid peroxidation with IC_{50} value 117.09 ± 2.01 µg/ml, 15.62 ± 1.31 µg/ml, and 66.606 ± 1.67 µg/ml respectively. From our results, it is clear that CLF possess potential lipid peroxidation inhibition activity as shown in table 2.

Discussions

Polyphenols are one of the most common sources of antioxidants. Therefore, plants with high content of polyphenol are very useful against neurodegenerative disorders [31]. A study similar to the present one was done by Sántos-Sanchez et al., on acetone fractions of the plant *Heliocarpus terebinthinaceae;* the result was 301.6 ± 12.3 mg of GAE/g of dried seed extract, which is higher than that of the *Grewia abutilifolia* extracts [32].

Flavonoids, a large family of natural antioxidants, undergo a significant hepatic metabolism leading to flavonoid-derived metabolites that are bioactive as well as antioxidant agents and can give neuroprotection. In a study of *Grewia asiatica* (Family: Tiliaceae), ethanolic extract of the leaf revealed that the total flavonoids present was 90.67 mg of QE/g of dry extract [31], which is much less than of our present study.

Flavonols preserve cognitive abilities during ageing in animals, lower the risk of developing Alzheimer's disease and decrease the risk of stroke in humans. This study revealed greater value than the previous study of *Morus alba* (Family: Moraceae) by the same method [33].

Recent research shows that extracts rich in proanthocyanidins exhibited greater neuroprotective activity than extracts rich in other polyphenols [34]. These proanthocyanidins have been shown to protect brain cells that contain dopamine from being damaged or dying as a result of oxidative stress. Compared to a previous study of the plant *Morus alba* (Family: Moraceae), our study revealed better results [35].

In the extracts, it was found that the total antioxidant activity increased with the increasing concentration of the extract. The previous study on *Morus alba* (Family: Moraceae) showed less absorbance than the present study [35].

Among the four fractions, CLF showed most significant Fe^{3+} reducing power capacity. This capacity increased with increasing concentration of the extract and these results indicated that CLF has potential iron reducing capacity; however, it was less than *Morus alba* (Family: Moraceae), which was studied previously by the same method [35].

Reactive oxygen species (ROS) production and oxidative damage to biomacromolecules (nucleic acids, lipids and proteins) can represent a suitable environment for the development of neurological diseases. Therefore, radical scavenging

activities are one of the most common approaches to combat neurological as well as age related diseases [20]. Free radical scavenging activity of plant *Heliocarpus terebinthinaceae* acetone leaf fraction was 1970 ± 3 [32], which much lower than the extractive of *Grewia abutilifolia*.

Hydroxyl radicals are the major reactive oxygen species causing lipid oxidation; enormous biological damages have been incurred due to hydroxyl radicals [36]. In our study, *Grewia abutilifolia* extract showed greater activity than the hydroxyl radical scavenging activity of methanolic fruit extract of *Phyllanthus emblica* L., which was observed in a similar way [37].

Oxidative damage in neurodegenerative disorders predominantly manifests as lipid peroxidation; increased lipid peroxidation has been observed in the brain of neurological patients [20,34,35]. In comparison with the lipid peroxidation inhibition activity of methanolic fruit extract of *Phyllanthus emblica* L., which was performed in an earlier study, *Grewia abutilifolia* has considerable inhibitory activity against lipid peroxidation [37].

Conclusion

This study results suggest that the crude methanol extract and other fractions obtained from methanolic extract of leaves of *Grewia abutilifolia* contained significant quantity of polyphenols, flavonols, proanthocyanidins, and flavonoids. These compounds have also shown significant antioxidants. Due to the presence of significant chemicals with antioxidant activity, this plant could be a potential source of compounds for the development of drugs which may prevent diseases caused by oxidative stress.

Submission Declaration

I hereby affirm that the content of this manuscript is original. Furthermore, it has been neither published elsewhere fully or partially or in any language nor submitted for publication (fully or partially) elsewhere simultaneously. I also affirm that all the authors have seen and agreed to the submission of paper and their inclusion of names as co-authors.

Author's contributions

RS and MRR both performed the laboratory tests and prepared the manuscript. MRR performed literature review and collected the plant materials. RS designed, supervised and interpreted the results of the study.

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Competing interests: the authors declare that they have no competing interests.

Conflicts of interests: the authors have no conflicts of interests.

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