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Phytochemical Screening Ethanol Extract of Barks in *Gmelina Arborea* and *Grewia Umbellifera*

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Abstract: *In this paper focused on the The medicinal plants GA and GU confirmed the presence of many phytoconstituents present in the ethanol extract showed a significant number of phytochemicals in them and the ethanol extract were used for further studies, The spectral studies of the plants also revealed the presence of many components in them. The study on in vitro antioxidant activity of medicinal plants also revealed the antioxidant potential of the plants. Hepatoprotective activity studies of the barks of GA and GU on paracetamol induced rats showed the significant protective activity.The ethanol extract of GA and GU extracts showed significant nephroprotective activity.In conclusion, the results of the present investigation infer that these plants (GA and GU) extracts possess potent antioxidant, Hepatoprotective and nephroprotective property, the former being probably responsible than the later. Thus, the extracts can be beneficial in treating liver and renal damages caused due to chemical exposure.*

Keywords: *Gmelina arborea, Ggrewia umbellifera, borntrager's test.*

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I. Introduction

Medicinal plants play a key role in the human health care. About 80% of the world population rely on the use of traditional medicine which is predominantly based on plant materials. The traditional medicine refers to a broad range of ancient natural health care practices including folk/tribal practices as well as Ayurveda, Siddha, Amchi and Unani. These medical practices originated from time immemorial and developed gradually, to a large extent, by relying or based on practical experiences without significant references to modern scientific principles. It is estimated that about 7500 plants are used in local health traditions in, mostly, rural and tribal villages of India. Out of these, the real medicinal value of over 4000 plants is either little known or hitherto unknown to the mainstream population. The classical systems of medicine such as Ayurveda, Siddha, Amchi, Unani and Tibetan use about 1200 plants. A detailed investigation and documentation of plants used in local health traditions and pharmacological evaluation of these plants and their taxonomical relatives can lead to the development of invaluable plant drugs for many dreaded diseases. Random screening of plants has not proved economically effective.

II. Collection And Identification Of Plants

The bark of GA and GU were collected from south India, Kanyakumari district during the month of January and Febuary. The plant was identified by S. Balasubramanium, ABS Botanical Garden - Salem.

III. Preparation Of Extracts

The freshly collected barks were dried in shade, then coarsely powdered. For extraction of crude phytochemical, 25 g of powdered bark material was kept in closed conical flask with 20 mL various solvents like petroleum ether, benzene, chloroform, ethanol, acetone, ethyl acetate and distilled water in a shaker at room temperature for 24 h. After incubation, the extracts were filtered and the extracts were collected and stored in the refrigerator at 4 for further studies. All the extracts were subjected to preliminary phytochemical screening as per the guidelines.

IV. Materials And Methods

IV.I. PRELIMINARY PHYTOCHEMICAL SCREENING OF BARK EXTRACT OF *GREWIA UMBELLIFERA* AND *GMELINA ARBOREA*

The extracts obtained as above were then subjected to qualitative tests for the identification of various plant constituents.

V. Detection Of Carbohydrates

A minimum amount of extracts were suspended in 5 ml of distilled water. The suspension was subjected to General test, Starch test, Barfoed's test, Molisch's test, Fehling's test, Benedict's test, Iodine test as seen below.

a) General Test

The extracts were treated with a few ml of distilled water and sulphuric acid.
Formation of dull violet precipitate indicates the presence of reducing sugar.

b) Starch Test

Aqueous extracts were treated with 5ml of 5% potassium hydroxide. Canary coloured solution shows the presence of starch.

c) Barfoed's test

Aqueous extracts were treated with 1ml of Barfoed's reagent. The solutions were heated in a beaker of boiling water bath gives a red precipitate indicates the presence of reducing sugar.

d) Molisch's Test

The extracts were treated with 2-3 drops of 1% alcoholic alpha napthol and 2ml of concentrated sulphuric acid was added along the sides of the test tube. The formation of purple ring between two layers shows the presence of carbohydrates.

e) Fehling's Test

The extracts were treated with Fehling's A and B solution and heated for few minutes. Formation of brick red precipitate shows the presence of reducing sugar.

f)Benedict's Test

The extracts were treated with Benedict's reagent and heated for few minutes.
Formation of red precipitate shows the presence of reducing sugar.

g) Iodine Test

Add a few drops of iodine solution to 1ml of the extract. Formation of deep blue colour indicates the presence of starch.

VI. Detection Of Glycosides

Minimum quantities of the extracts were hydrolyzed with hydrochloric acid for few minutes on a water bath and the hydrolyzate was subjected to Legal's test, Bontrager's test, Ferric Chloride's test as seen below.

a) Legal's Test

To the hydrolyzate 1ml of pyridine and few drops of sodium nitro prusside solution were added and then it was made alkaline with sodium hydroxide. The pink colour changes in to red show the presence of glycosides.

b) Borntrager's Test

Hydrozylate was treated with chloroform and the chloroform layer was separated. To this equal quantity of dilute ammonia solution was added. The pink colour changes in to red show the presence of glycosides.

c) Ferric chloride Test

2ml of extracts were treated with 1ml of glacial acetic acid and 1ml of ferric chloride. Also add few drops of concentrated sulphuric acid. Formation of blue colour showed the presence of glycosides.

VII. Detection Of Proteins And Amino Acids

A small quantity of extract was dissolved in few ml of water and they were subjected to Million's test, Ninhydrin test, Biuret test as given below.

a) Million's Test

The extracts were treated with Millon's reagent. The precipitate was formed with the extract, which shows the presence of proteins.

b) Ninhydrin test

The extracts were treated with Ninhydrin reagent. The purple colour was formed with extract, which shows the presence of proteins.

c) Biuret Test

To the extracts equal volume of 5% sodium hydroxide solution and 1% copper solutions were added. A violet colour formation indicates the presence of amino acids.

VIII. Detection Of Fixed Oils And Fats

A small quantity of extract was subjected to Spot test, Saponification test as follows.

a) Spot Test

Small quantities of extracts were placed between two filter papers. The production of stains with alcoholic extract shows the presence of fats and fixed oils in the extract.

b) Saponification Test

Few drops of 0.5N alcoholic potassium hydroxide was added to the extracts with few drops on phenolphthalein solution. Later the mixture was heated on a water bath for 1-2 hours. The soap formation indicates the presence of fat and fixed oils in the alcoholic extracts.

IX. Detection Of Alkaloids

A small quantity of the extracts were treated with few drops of dilute hydrochloric acid and filtered. The filtrate was tested with alkaloid reagent such as Mayer's reagent, Dragondroff's reagent, Hager's reagent, Wagner's reagent and subjected to potassium dichromate test as given below.

The filtrate was tested with alkaloid reagent such as,

1) Mayer's reagent (Cream precipitate)
2) Dragondroff's reagent (Reddish brown precipitate)
3) Hager's reagent (Yellow precipitate)
4) Wagner's reagent (Reddish brown precipitate)
5) Potassium dichromate Test

The extracts were treated with concentrated sulphuric acid and add small amount of potassium dichromate. No colour change indicates the presence of indole alkaloid.

X. Detection Of Flavanoids

i) Ferric chloride Test

Aqueous extracts were treated with few drops of 10% ferric chloride. Formation of green precipitate indicates the presence of Flavanoids.

ii) Lead acetate Test

Aqueous extracts were treated with few ml of 10% lead acetate. Buff coloured solution formation indicates the presence of Flavanoids.

iii) Test for Anthraquinone

a) 0.5gm of extract was treated with 10ml of sulphuric acid. This solution was boiled and filtered while hot. From the filtrate add 5ml of chloroform. Pipetted out the formed chloroform layer add 1ml of dilute ammonia. No colour change indicates the presence of Anthraquinone.
b) 0.5gm of Anthroquinone added a drop of benzene and ammonia. Formation of pink colour indicates the presence of Anthraquinone.

iv) Test for Catechins

A drop of Erlich's reagent was added to the 0.5gm of extracts. Formation of pink colour indicates the presence of catechins.

v) Test for Anthocyanin

2ml of plant extracts were treated with 1ml of 2M NaOH and heated for 5 min. Formation of yellow colour indicates the presence of Anthocyanin.

XI. Detection Of Phytosterols

Small quantities of extracts were suspended in 5ml of chloroform separately.

The above obtained chloroform solution was subjected to Libermann Burchard test,

Salkowski test as given below.

a) Libermann Burchard Test

The above prepared chloroform solutions were treated with few drops of concentrated sulphuric acid. A bluish green solution indicates the presence of phytosterols.

b) Salkowski Test

To the above prepared chloroform solutions, a few drops of concentrated sulphuric acid were added. Formation of brown ring with chloroform extract indicates the presence of phytosterols.

XII. Detection Of Tannins- Phenolic Compounds

All the extracts were dissolved or suspended separately in minimum amount of water and filtered. The filtrate was subjected to General test, Ferric Chloride test, Lead acetate test and Phlonatannins as given below.

a) General Test

Plant extracts were treated with a few drops of sulphuric acid and 1 drop of 5% HCl. Formation of green colour indicates the presence of tannins.

b) Ferric chloride Test

To the filtrates few drops of ferric chloride was added. Violet colour precipitate indicates the presence of tannins.

d) Test for Phlonatannins

The ethanol extract of plant material was treated with 5ml of 1% hydrochloric acid. Formation of red precipitate indicates the presence of phlonatannins.

XIII. Detection Of Saponins

The extracts were subjected to Foam test, Haemolysis test, as seen below.

a) Foam Test

The extract was diluted with 20ml of distilled water and then agitated in a graduated cylinder for 15 minutes. A 1cm layer of foam indicates the presence of Saponins.

b) Haemolysis Test

About 2ml of blood was taken two test tubes separately. To one of the test tubes, equal quantity of water was added. To the other test tube, an equal quantity of ethanolic extract dissolved in water wad added. A clear red liquid was formed in the first test tube, which indicates the red blood corpuscles were haemolysed. The extract in the second tube also haemolysed. It indicates the presence of Saponins.

XIV. Detection Of Steroids, Vitamins And Terpenoids

The extracts were subjected to various tests, as follows.

a)Detection of Steroids

To the ethanolic extract add few drops of acetic anhydride and a drop of concentrated sulphuric acid. Appearance of green or brown colour was the end point.

b)Detection of Vitamins

One or two drops of plant extracts were treated with 1ml of chloroform and a drop of concentrated sulphuric acid. A colour changes from violet to brown colour indicates the presence of vitamins.

c)Detection of Terpenoids

Aqueous extract of plant materials were treated with 2ml of chloroform and few drops of concentrated sulphuric acid. Formation of reddish brown colour at interphase indicates the presence of terpenoids.

d) *In vitro* antioxidant studies of barks of *Gmelina arborea* and *Grewia umbelliferea*

The efficacy of the ethanol extract of barks of *Gmelina arborea* and *Grewia umbelliferea* was studied under *in vitro* conditions. The free radical scavenging activity barks of *Gmelina arborea* and *Grewia umbelliferea* against 1, 1-diphenyl-2-picrylhydrazyl (DPPH), 2, 2'-azino-bis (3-ethylbenzthiazoline -6-sulfonic acid) (ABTS), nitric oxide and hydrogen peroxide, super oxide, lipid peroxidation, reducing potential, hydroxyl radical scavenging were also studied.

e)DPPH Radical Scavenging Activity

DPPH radical scavenging activity was carried out by the method of Molyneux (2004). To 1 ml of 100 µM DPPH solution in methanol, equal volume of the test sample in methanol of different concentration was

added and incubated in dark for 30 minutes. The change in colouration was observed in terms of absorbance using a spectrophotometer at 514 nm. 1 ml of methanol instead of test sample was added to the control tube. Different concentration of ascorbic acid was used as reference compound. Percentage of inhibition was calculated from the equation:

$$\left(\frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}}\right) \times 100$$

IC50 value was calculated using Graph pad prism 5.0.

f) ABTS Radical Scavenging Activity

ABTS radical scavenging activity was performed as described by **Re *et al.*,(1999)** with a slight modification. 7 mM ABTS in 14.7 mM ammonium peroxo-disulphate was prepared in 5 ml distilled water. The mixture was allowed to stand at room temperature for 24 hours. The resulting blue green ABTS radical solution was further diluted such that its absorbance is 0.70±0.02 at 734 nm. Various concentration of the sample solution dissolved in ethanol (20 µl) was added to 980 µl of ABTS radical solution and the mixture was incubated in darkness for 10 min. The decrease in absorbance was read at 734 nm. A test tube containing 20 µl of ethanol and processed as described above served as the control tube. Different concentration of ascorbic acid was used as reference compound.

g)Hydrogen Peroxide Radical Scavenging Activity

Hydrogen peroxide radical scavenging activity of the test sample was estimated by the method of **(Ruch *et al.*, 1989)**. A solution of hydrogen peroxide was prepared in phosphate buffer (pH 7.4). 200 µl of sample containing different concentration was mixed with 0.6 ml of H2O2 solution. Absorbance of H2O2 was determined 10 minutes later against a blank solution containing phosphate buffer without H2O2. A test tube containing 200 µl of phosphate buffer and processed as described above served as the control tube. Different concentration of ascorbic acid was used as reference compound.

h)Superoxide Radical Scavenging Activity

Superoxide radical scavenging activity of the test sample was studied by the method of with slight modifications. Superoxide radicals are generated in phenazine methosulphate (PMS) - Nicotinamide adenine dinucleotide (NADH) systems by oxidation of NADH and assayed by the reduction of Nitro Blue Tetrazolium (NBT). 200 µl of test sample of different concentration was taken in a series of test tubes. Superoxide radicals were generated by 1 ml of Tris-HCl buffer (16 mM, pH-8), 1 ml of NBT (50 µM), 1 ml NADH (78 µM) solution and 1 ml of PMS (10 µM). The reaction mixture was incubated at 25°C for 5 min and the absorbance at 560 nm was measured. A control tube containing Tris-HCl buffer was also processed in the same way without test sample. Different concentration of ascorbic acid was used as reference compound.

i)Hydroxyl Radical Scavenging Activity

The hydroxyl radical scavenging activity of the test sample was estimated according to the method of **Halliwell *et al.*, (1992)**. The hydroxyl radical was generated by a fenton-type reaction. The reaction mixture contained 0.2 ml of sample in varied concentration to which, 0.1 ml EDTA (1 mM)-FeCl3 (10 mM) mixture, 0.1 ml H2O2 (10 mM), 0.36 ml deoxyribose (10 mM), 0.33 ml phosphate buffer (50 mM, pH 7.4) and 0.1 ml of ascorbic acid (1 mM) was added in sequence. The mixture was incubated at 37°C for 1 h. To this mixture was added 1.0 ml each of TCA (10 %) and TBA (0.67 %) and kept in boiling water bath for 20 minutes. The colour developed was read at 532 nm. The control tube contains phosphate buffer, instead of sample. Different concentration of ascorbic acid was used as reference compound.

J)Total Reducing Potential

The total reducing potential of the different fractions were screened using the method of **Oyaizu (1986)**. 0.75 ml of the sample at various concentrations was mixed with 0.75 ml of phosphate buffer (0.2 M, pH 6.6) and 0.75 ml of 1 % potassium ferricyanide and incubated at 50°C in a water bath for 20 min. The reaction was stopped by the addition of 0.75 ml of 10 % TCA solution and then, centrifuged at 800 g for 10 min. 1.5 ml of the supernatant was mixed with 1.5 ml of distilled water and 0.1 ml of 0.1 % ferric chloride and kept at room temperature for 10 min. The absorbance was read at 700 nm. The values are expressed as ascorbic acid equivalence.

k)Nitric Oxide Radical Scavenging

Nitric oxide radical scavenging activity was measured spectrophotometrically by the method of **Govindharajan *et al*., (2003)**. 1.0 ml of sodium nitroprusside (5 mM) in phosphate buffer (p H 7.4, 0.1 M) was mixed with different concentration of the extract (100–500 μg/ml in phosphate buffer (pH 7.4, 0.1 M). The tubes were then incubated at 25^{o}C for two hours. At the end of second hour 1.5 ml of reaction mixture was removed and diluted with 1.5 ml of Greiss reagent (1% sulphanilic acid, 3% o-phosphoric acid, 0.1% of naphthyl ethylenediamine dihydrochloride). The absorbance of the chromophore formed during diazotization of the nitrite with sulphanilic acid and subsequent coupling with naphtylethylene diamine dihydrochloride was measured at 546 nm. Control tube contain all chemicals except plant extract.

XV. Result And Discussion

Table: 1 Phytochemical screening of bark extract of *Gmelina arborea*

S. No	Name of the test	***Gmelina arborea* bark extracts**						
		Pet. ether	**Benzene**	**Chloroform**	**Ethanol**	**Acetone**	**Ethyl acetate**	**Dist. water**
1	**CARBOHYDRATES**							
	a) General test	+	+	+	+++	+	+	+
	b) Starch test	+	+	+	+++	+	+	+
	c) Barfoed's test	+	+	+	+++	+	+	+
	d) Molisch's test	+	+	+	+++	+	+	+
	e) Fehling's test	+	+	+	+++	+	+	+
	f) Benedict's test	+	+	+	+++	+	+	+
	g) Iodine test	+	+	+	+++	+	+	+
2	**DETECTION OF GLYCOSIDES**							
	a) Legal' test	+	+	+	++	+	+	+
	b)Borntrager's test	+	+	+	++	+	+	+
	c) Ferric chloride test	+	+	+	++	+	+	+
3	**PROTEINS AND AMINO ACIDS**							
	a) Million's test	-	+	+	++	+	-	-
	b) Ninhydrin test	-	+	+	++	+	-	-
	c) Biuret test	-	+	+	++	+	-	-
4	**FIXED OILS AND FATS**							
	a) Spot test	+	+	-	++	+	+	+

	b)Saponificati-on test	+	+	-	++	+	+	+
5	**DETECTION OF ALKALOIDS**							
	a) Mayer's test	-	+	-	+++	+	+	-
	b)Dragondroff-'s test	-	+	-	+++	+	+	-
	c) Hager's test	-	+	-	+++	+	+	-
	d) Wagner's test	-	+	-	+++	+	+	-
	e) Potassium dichromate test	-	+	-	+++	+	+	-
6	**DETECTION OF FLAVANOIDS**							
	a) Ferric chloride test	+	+	+	++	+	+	+
	b) Lead acetate test	+	+	+	++	+	+	+
	c) Test for Anthraquinone	+	+	+	++	+	+	+
	d) Test for Catechins	+	+	+	++	+	+	+
	e) Test for Anthocyanin	+	+	+	++	+	+	+
7	**DETECTION OF PHYTOSTEROLS**							
	a) Libermann Burchard test	+	-	-	+	+	+	-
	b) Salkowski test	+	-	-	+	+	+	-
8	**TANNINS AND PHENOLIC COMPOUNDS**							
	a) General test	-	+	+	+	+	+	+
	b) Ferric chloride test	-	+	+	+	+	+	+

	c) Test for Phlonatannins	-	+	+	+	+	+	+
9	**DETECTION OF SAPONINS**							
	a) Foam test	-	-	-	+	+	-	-
	b) Haemolysis test	-	-	-	+	+	-	-
10	DETECTION OF STEROIDS	-	-	-	+	+	-	-
11	DETECTION OF VITAMINS	-	-	-	+	+	-	-
12	DETECTION OF TERPENOIDS	-	-	-	+	+	-	-

Table: 2 Preliminary phytochemical screening of bark extract of *Grewia umbellifera*

S. No	Name of the test	***Grewia umbellifera* bark extracts**						
		Pet. ether	**Benzene**	**Chloroform**	**Ethanol**	**Acetone**	**Ethyl acetate**	**Dist. water**
1	**CARBOHYDRATES**							
	a) General test	-	-	+	++	+	+	-
	b) Starch test	-	-	+	++	+	+	-
	c) Barfoed's test	-	-	+	++	+	+	-
	d) Molisch's test	-	-	+	++	+	+	-
	e) Fehling's test	-	-	+	++	+	+	-
	f) Benedict's test	-	-	+	++	+	+	-
	g) Iodine test	-	-	+	++	+	+	-
	DETECTION OF GLYCOSIDES							

2	a) Legal' test	-	-	+	+	+	+	-
	b) Borntrager's test	-	-	+	+	+	+	-
	c) Ferric chloride test	-	-	+	++	+	+	-
3	**PROTEINS AND AMINO ACIDS**							
	a) Million's test	-	+	+	++	+	-	-
	b) Ninhydrin test	-	+	+	++	+	-	-
	c) Biuret test	-	+	+	++	+	-	-
4	**FIXED OILS AND FATS**							
	a) Spot test	+	+	-	++	+	-	-
	b) Saponification test	+	+	-	++	+	-	-
5	**DETECTION OF ALKALOIDS**							
	a) Mayer's test	-	+	-	++	+	+	-
	b) Dragondroff's test	-	+	-	++	+	+	-
	c) Hager's test	-	+	-	++	+	+	-
	d) Wagner's test	-	+	-	++	+	+	-
	e) Potassium dichromate test	-	+	-	+++	+	+	-
6	**DETECTION OF FLAVANOIDS**							
	a) Ferric chloride test	-	-	+	+	+	-	-
	b) Lead acetate test	-	-	+	+	+	-	-
	c) Test for Anthraquinone	-	-	+	+	+	-	-
	d) Test for Catechins	-	-	+	+	+	-	-

	e) Test for Anthocyanin	-	-	+	+	+	-	-
7	**DETECTION OF PHYTOSTEROLS**							
	a) Libermann Burchard test	+	-	-	+	+	-	-
	b) Salkowski test	+	-	-	+	+	-	-
8	**TANNINS AND PHENOLIC COMPOUNDS**							
	a) General test	-	-	+	+	+	-	-
	b) Ferric chloride test	-	-	+	+	+	-	-
	c) Test for Phlonatannins	-	-	+	+	+	-	-
9	**DETECTION OF SAPONINS**							
	a) Foam test	-	-	-	+	+	-	-
	b) Haemolysis test	-	-	-	+	+	-	-
10	**DETECTION OF STEROIDS**	-	-	-	+	+	-	-
11	**DETECTION OF VITAMINS**	-	-	-	+	+	-	-
12	**DETECTION OF TERPENOIDS**	-	-	-	+	+	-	-

XVI. IN VITRO ANTIOXIDANT ASSAY OF ETHANOL EXTRACT OF BARKS OF GMELINA ARBOREA AND GREWIA UMBELLIFERA

Different concentrations ranging from 25-125 μg/ml of the ethanol extract of barks of *Gmelina arborea* and *Grewia umbellifera* were tested for their antioxidant activity in different *in vitro* models. The percentage of inhibition was observed and found that the free radicals were scavenged by the test compounds in a concentration dependent up to the given concentration in all the models.

a)DPPH Radical Scavenging Activity

The activity of DPPH radical scavenging of the bark extract of ethanol extract of barks of *Gmelina arborea* was presented in Table 3 and Figure 1. The percentage of inhibition in DPPH in different concentration like 25, 50, 75, 100, 125 μg/ml were observed in 35.52±2.05, 60.85±1.19, 75.21±1.89, 89.90±2.47 and 95.62±1.14 respectively where as the percentage inhibition of ascorbic acid in concentration like 25, 50, 75, 100, 125 μg/ml were found to be 27.31±0.98, 51.05±1.97, 63.71±1.31, 77.32±1.87 and 90.45±1.49 respectively. The IC50 values for DPPH scavenging activity for ethanol extract of barks of *Gmelina arborea* and ascorbic acid were 36.5 μg/ml and 43.5 μg/ml respectively. Values are the average of triplicate experiments and represented as mean ± standard deviation.

The activity of DPPH radical scavenging of the bark extract of ethanol extract of barks of *Grewia umbellifera* was presented in Table 3.1 and Figure 1.1. The percentage of inhibition in DPPH in different concentration like 25, 50, 75, 100, 125 µg/ml were observed in 22.18±1.32, 40.84±1.19, 55.25±1.89, 68.09±2.66 and 80.13±2.44 respectively whereas the percentage inhibition of ascorbic acid in concentration like 25, 50, 75, 100, 125 µg/ml were found to be 27.31±0.98, 51.05±1.97, 63.71±1.31, 77.32±1.87 and 90.45±1.49 respectively. The IC50 values for DPPH scavenging activity for bark extract of ethanol extract of barks of *Grewia umbellifera* and ascorbic acid were 48.5 µg/ml and 43.5 µg/ml respectively. Values are the average of triplicate experiments and represented as mean ± standard deviation.

Table: 3 DPPH radical scavenging activity of ethanol extract of barks of *Gmelina arborea*

Group	**Concentration (µg/ml)**	**% of Inhibition**	**IC50 Value**
Ethanol extract of barks of *Gmelina arborea*	25	35.52±2.05	36.5 µg/ml
	50	60.85±1.19	
	75	75.21±1.89	
	100	89.90±2.47	
	125	95.62±1.14	
Ascorbic Acid	25	27.31±0.98	43.5 µg/ml
	50	51.05±1.97	
	75	63.71±1.31	
	100	77.32±1.87	
	125	90.45±1.49	

Table: 3.1 DPPH radical scavenging activity of ethanol extract of barks of *Grewia umbellifera*

Group	**Concentration (µg/ml)**	**% of Inhibition**	**IC50 Value**
Ethanol extract of barks of *Grewia umbellifera*	25	22.18±1.32	48.5 µg/ml
	50	40.84±1.19	
	75	55.25±1.89	
	100	68.09±2.66	
	125	80.13±2.44	
Ascorbic Acid	25	27.31±0.98	43.5 µg/ml
	50	51.05±1.97	
	75	63.71±1.31	
	100	77.32±1.87	
	125	90.45±1.49	

Fig 1 Graphical representation of DPPH radical scavenging activity of ethanol extract of barks of *Gmelina arborea*

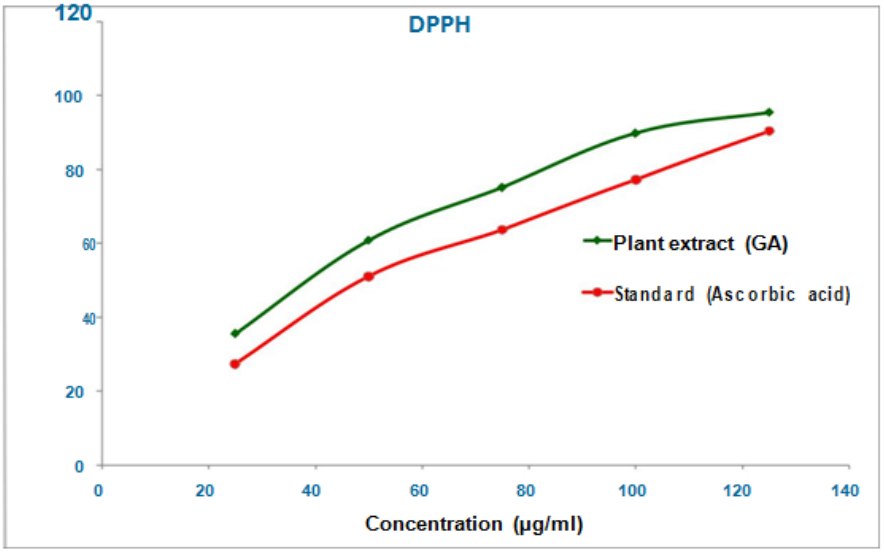
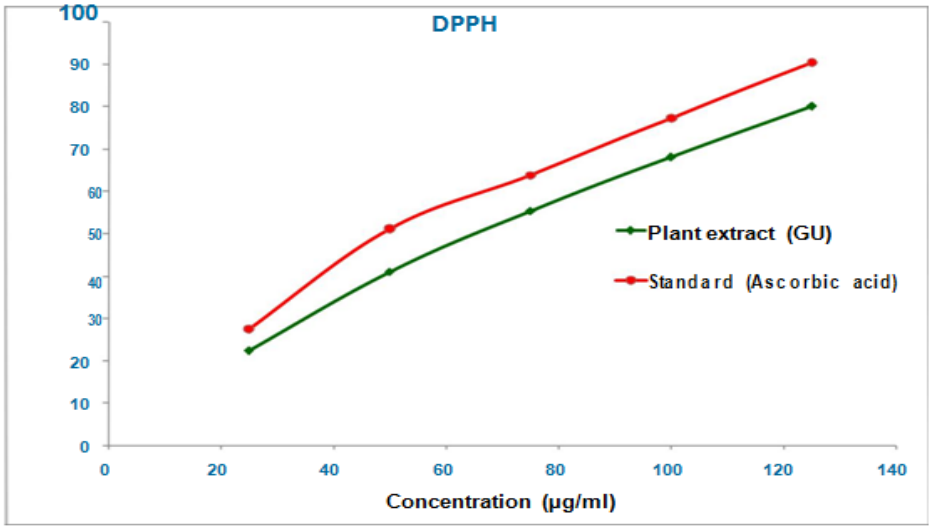


Fig 1.1 Graphical representation of DPPH radical scavenging activity of ethanol extract of barks of *Grewia umbellifera*



b)ABTS Radical Scavenging Activity

Table 4 and Fig 2 Shows the percentage of inhibition in ABTS in different concentration like 25, 50, 75, 100, 125 µg/ml were observed in 40.57±1.02, 60.53±1.13, 77.26±1.76, 88.07±2.07 and 93.47±2.36 respectively where as the percentage inhibition of ascorbic acid in concentration like 25, 50, 75, 100, 125 µg/ml were found to be 33.48±1.19, 49.12±1.89, 62.33±1.46, 77.63±0.91 and 89.78±0.66 respectively. The IC50 values for ABTS scavenging activity for ethanol extract of barks of *Gmelina arborea* and ascorbic acid were 32.5 µg/ml and 42.5 µg/ml respectively. Values are the average of triplicate experiments and represented as mean ± standard deviation.

Table 4.1 and Fig 2.1 Shows the percentage of inhibition in ABTS in different concentration like 25, 50, 75, 100, 125 µg/ml were observed in 28.90±1.95, 41.32±1.13, 52.19±1.76, 69.48±2.07 and 77.96±2.21 respectively where as the percentage inhibition of ascorbic acid in concentration like 25, 50, 75, 100, 125 µg/ml were found to be 33.48±1.19, 49.12±1.89, 62.33±1.46, 77.63±0.91 and 89.78±0.66 respectively. The IC50 values for ABTS scavenging activity for ethanol extract of barks of *Grewia umbellifera* and ascorbic acid were 45.5 µg/ml and 42.5 µg/ml respectively. Values are the average of triplicate experiments and represented as mean ± standard deviation.

Table :4 ABTS radical scavenging activity of ethanol extract of barks of *Gmelina arborea*

Group	Concentration (µg/ml)	% of Inhibition	IC50 Value
Ethanol extract of barks of *Gmelina arborea*	25	40.57±1.02	32.5 µg/ml
	50	60.53±1.13	
	75	77.26±1.76	
	100	88.07±2.07	
	125	93.47±2.36	
Ascorbic Acid	25	33.48±1.19	42.5 µg/ml
	50	49.12±1.89	
	75	62.33±1.46	
	100	77.63±0.91	
	125	89.78±0.66	

Table: 4.1 ABTS radical scavenging activity of ethanol extract of barks of *Grewia umbellifera*

Group	Concentration (µg/ml)	% of Inhibition	IC50 Value
Ethanol extract of barks of *Grewia umbellifera*	25	28.90±1.95	45.5 µg/ml
	50	41.32±1.13	
	75	52.19±1.76	
	100	69.48±2.07	
	125	77.96±2.21	
Ascorbic Acid	25	33.48±1.19	42.5 µg/ml
	50	49.12±1.89	
	75	62.33±1.46	
	100	77.63±0.91	
	125	89.78±0.66	

Fig 2 Graphical representation of ABTS activity of ethanol extract of barks of *Gmelina arborea*

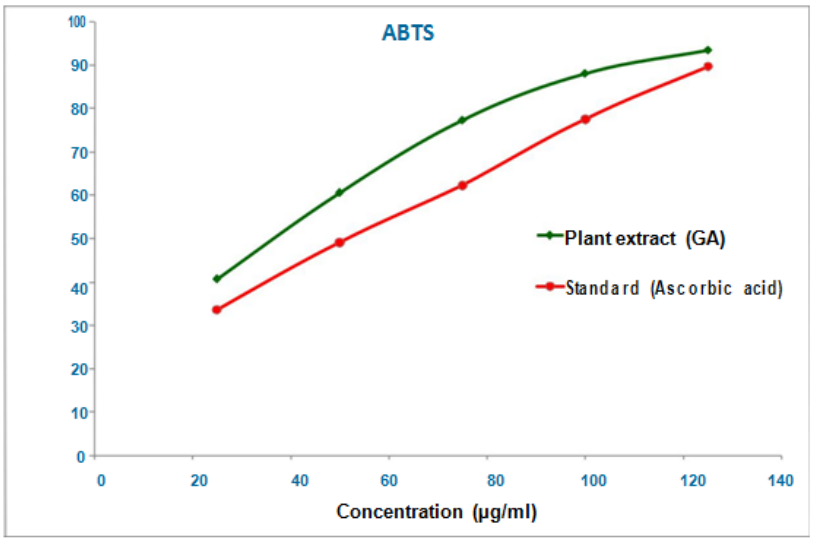


Fig 2.1 Graphical representation ABTS activity of ethanol extract of barks of *Grewia umbellifera*

c) Hydrogen Peroxide Radical Scavenging Activity

Table 5 and Figure 3 shows the percentage of inhibition of H2O**2** in different concentration like 25, 50, 75, 100, 125 µg/ml were observed in 35.51±1.20, 54.62±2.14, 69.05±2.21, 89.41±1.96 and 96.48±2.52 respectively whereas the percentage inhibition of ascorbic acid in concentration like 25, 50, 75, 100, 125 µg/ml were found to be 26.85±0.92, 41.40±1.69, 59.85±1.54, 78.61±1.87 and 89.92±1.62 respectively. The IC50 values for hydrogen peroxide scavenging activity for ethanol extract of barks of *Gmelina arborea* and ascorbic acid were 42 µg/ml and 55 µg/ml respectively. Values are the average of triplicate experiments and represented as mean ± standard deviation.

Table 5.1 and Figure 3.1 shows the percentage of inhibition of H2O**2** in different concentration like 25, 50, 75, 100, 125 µg/ml were observed in 21.08±1.52, 30.47±2.14, 42.98±2.51, 65.72±1.81 and 80.44±2.02 respectively whereas the percentage inhibition of ascorbic acid in concentration like 25, 50, 75, 100, 125 µg/ml were found to be 26.85±0.92, 41.40±1.69, 59.85±1.54, 78.61±1.87 and 89.92±1.62 respectively. The IC50 values for hydrogen peroxide scavenging activity for ethanol extract of barks of *Grewia umbellifera* and ascorbic acid were 70 µg/ml and 55 µg/ml respectively. Values are the average of triplicate experiments and represented as mean ± standard deviation.

Table: 5 H2O2 radical scavenging activity of ethanol extract of barks of *Gmelina arborea*

Group	**Concentration (µg/ml)**	**% of Inhibition**	**IC50 Value**
Ethanol extract of barks of *Gmelina Arborea*	25	33.51±1.20	42 µg/ml
	50	54.62±2.14	
	75	69.05±2.21	
	100	89.41±1.96	
	125	96.48±2.52	
Ascorbic Acid	25	26.85±0.92	55 µg/ml
	50	41.40±1.69	
	75	59.85±1.54	
	100	78.61±1.87	
	125	89.92±1.62	

Table: 5.1 H2O2 radical scavenging activity of ethanol extract of barks of *Grewia umbellifera*

Group	**Concentration (µg/ml)**	**% of Inhibition**	**IC50 Value**
Ethanol extract of barks of *Grewia umbellifera*	25	21.08±1.52	70 µg/ml
	50	30.47±2.14	
	75	42.98±2.51	
	100	65.72±1.81	
	125	80.44±2.02	
Ascorbic Acid	25	26.85±0.92	55 µg/ml
	50	41.40±1.69	
	75	59.85±1.54	
	100	78.61±1.87	
	125	89.92±1.62	

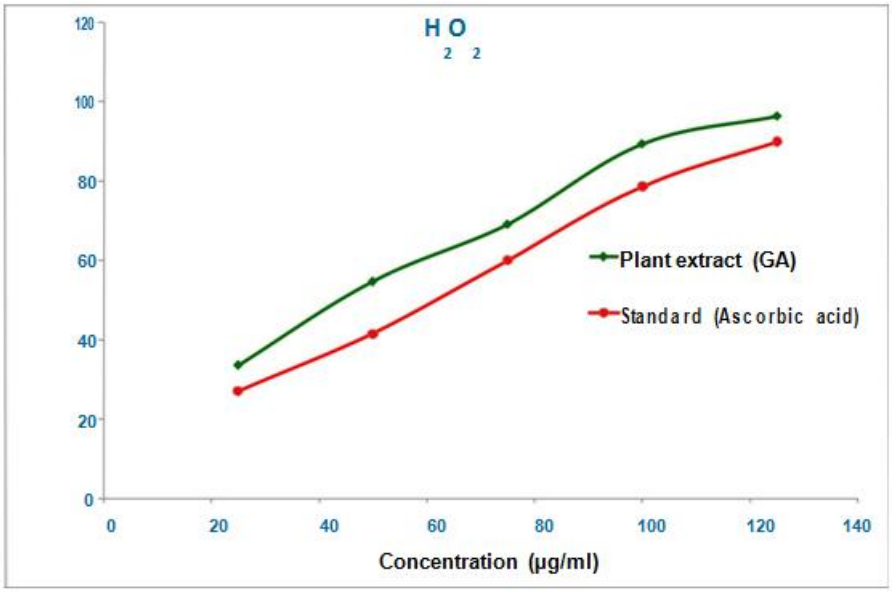


Fig 3 Graphical representation of H2O2 radical scavenging activity of ethanol extract of barks of *Gmelina arborea*

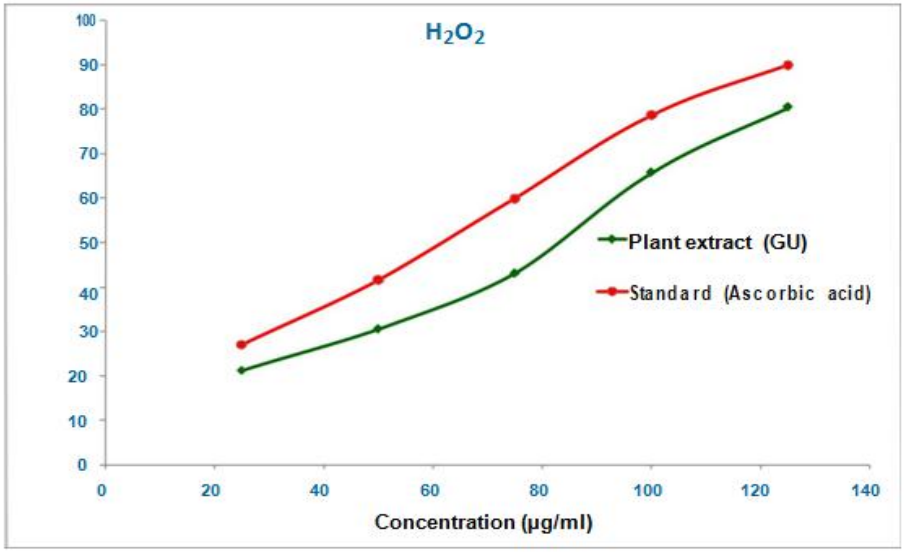


Fig 3.1Graphical representation of H2O2 radical scavenging activity of ethanol extract of barks of *Grewia umbellifera*

d)Superoxide Radical Scavenging Activity

Superoxide free radicals scavenged in different concentration like 25, 50, 75, 100, 125 µg/ml were observed in 38.90±1.56, 60.38±1.11, 82.62±2.44, 91.89±0.54 and 98.47±1.80 respectively where as the percentage inhibition of ascorbic acid in concentration like 25, 50, 75, 100, 125 µg/ml were found to be 30.56±1.21, 47.75±1.66, 69.51±2.09, 83.32±1.97 and 94.56±1.63 respectively. The IC50 values for superoxide scavenging activity for ethanol extract of barks of *Gmelina arborea* and ascorbic acid were 38 µg/ml and 39 µg/ml respectively which was presented in Table 6 and Figure

4. Values are the average of triplicate experiments and represented as mean ± standard deviation.

Superoxide free radicals scavenged in different concentration like 25, 50, 75, 100, 125 µg/ml were observed in 24.78±1.67, 39.44±1.11, 57.62±2.44, 70.13±0.54 and 81.21±1.80 respectively where as the percentage inhibition of ascorbic acid in concentration like 25, 50, 75, 100, 125 µg/ml were found to be 30.56±1.21, 47.75±1.66, 69.51±2.09, 83.32±1.97 and 94.56±1.63 respectively. The IC50 values for superoxide scavenging activity for ethanol extract of barks of *Grewia umbellifera* and ascorbic acid were 51 µg/ml and 39 µg/ml respectively which was presented in Table 6.1 and Figure 4.1. Values are the average of triplicate experiments and represented as mean ± standard deviation.

Table: 6 Superoxide radical scavenging activity of ethanol extract of barks of *Gmelina arborea*

Group	Concentration (µg/ml)	% of Inhibition	IC50 Value
Ethanol extract of barks of *Gmelina arborea*	25	38.90±1.56	38 µg/ml
	50	60.38±1.11	
	75	82.62±2.44	
	100	91.89±0.54	
	125	98.47±1.80	
Ascorbic Acid	25	30.56±1.21	39 µg/ml
	50	47.75±1.66	
	75	69.51±2.09	
	100	83.32±1.97	
	125	94.56±1.63	

Table 6.1 Superoxide radical scavenging activity of ethanol extract of barks of *Grewia umbellifera*

Group	Concentration (µg/ml)	% of Inhibition	IC50 Value
Ethanol extract of barks of *Grewia umbellifera*	25	24.78±1.67	51 µg/ml
	50	39.44±1.11	
	75	57.62±2.44	
	100	70.13±0.54	
	125	81.21±1.80	
Ascorbic Acid	25	30.56±1.21	39 µg/ml
	50	47.75±1.66	
	75	69.51±2.09	
	100	83.32±1.97	
	125	94.56±1.63	

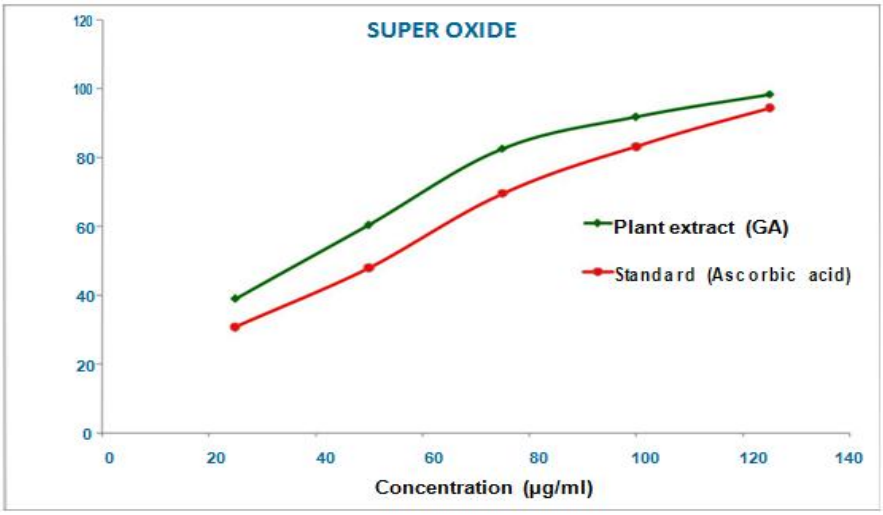


Fig 4 Graphical representation of Super oxide radical scavenging activity of ethanol extract of barks of Gmelina arborea

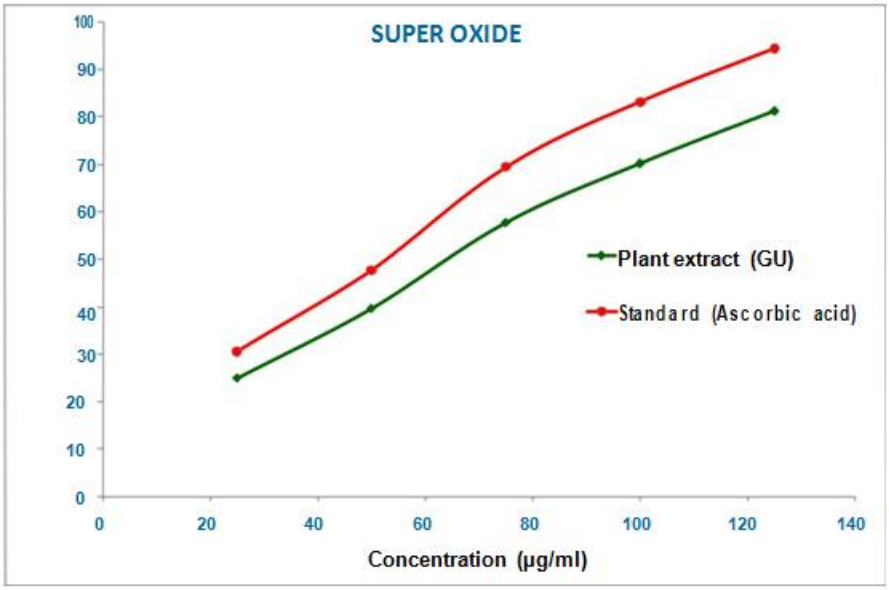


Fig 4.1 Graphical representation of Super oxide radical scavenging activity of ethanol extract of barks of *Grewia umbellifera*

e) Hydroxyl Radical Scavenging Activity

The hydroxyl radical scavenging activity of plant extract was presented in Table

7 and Figure 5 Hydroxyl radicals were scavenged in different concentration like 25, 50, 75, 100, 125 µg/ml were observed in 42.52±1.25, 63.90± 2.07, 78.39±1.45,

89.60±1.57 and 95.81±2.19 respectively where as the percentage inhibition of ascorbic acid in concentration like 25, 50, 75, 100, 125 µg/ml were found to be 31.95±1.97, 52.70±1.35, 65.40±1.62, 79.20±2.16 and 88.85±2.03 respectively. The IC50 values for hydroxyl radical scavenging activity of ethanol extract of barks of *Gmelina arborea* and ascorbic acid were 32 µg/ml and 39 µg/ml respectively. Values are the average of triplicate experiments and represented as mean ± standard deviation.

The hydroxyl radical scavenging activity of plant extract was presented in Table 7.1 and Figure 5.1. Hydroxyl radicals were scavenged in different concentration like 25, 50, 75, 100, 125 µg/ml were observed in 23.05±1.89, 40.11±1.53, 54.62±1.65, 65.89±0.98 and 80.16±1.21 respectively whereas the percentage inhibition of ascorbic acid in concentration like 25, 50, 75, 100, 125 µg/ml were found to be 31.95±1.97, 52.70±1.35, 65.40±1.62, 79.20±2.16 and 88.85±2.03 respectively. The IC50 values for hydroxyl radical scavenging activity of ethanol extract of barks of *Grewia umbellifera* and ascorbic acid were 50 µg/ml and 39 µg/ml respectively. Values are the average of triplicate experiments and represented as mean ± standard deviation.

Table: 7 Hydroxyl radical scavenging activity of ethanol extract of barks of *Gmelina arborea*

Group	**Concentration (µg/ml)**	**% of Inhibition**	**IC50 Value**
Ethanol extract of barks of *Gmelina arborea*	25	42.52±1.25	32 µg/ml
	50	63.90± 2.07	
	75	78.39±1.45	
	100	89.60±1.57	
	125	95.81±2.19	
Ascorbic Acid	25	31.95±1.97	39 µg/ml
	50	52.70±1.35	
	75	65.40±1.62	
	100	79.20±2.16	
	125	88.85±2.03	

Table 7.1 Hydroxyl radical scavenging activity of ethanol extract of barks of *Grewia umbellifera*

Group	Concentration (µg/ml)	% of Inhibition	IC50 Value
Ethanol extract of barks of *Grewia umbellifera*	25	23.05±1.89	50 µg/ml
	50	40.11±1.53	
	75	54.62±1.65	
	100	65.89±0.98	
	125	80.16±1.21	
Ascorbic Acid	25	31.95±1.97	39 µg/ml
	50	52.70±1.35	
	75	65.40±1.62	
	100	79.20±2.16	
	125	88.85±2.03	

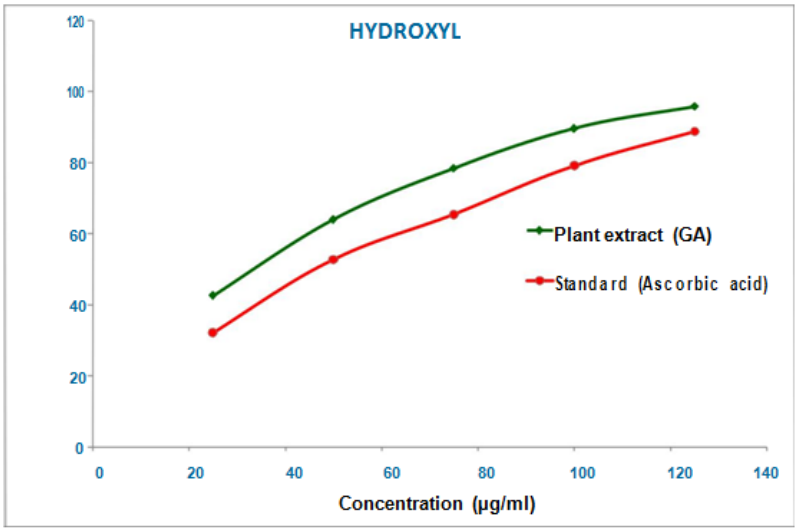


Fig 5 Graphical representation of Hydroxyl radical scavenging activity of ethanol extract of barks of *Gmelina arborea*

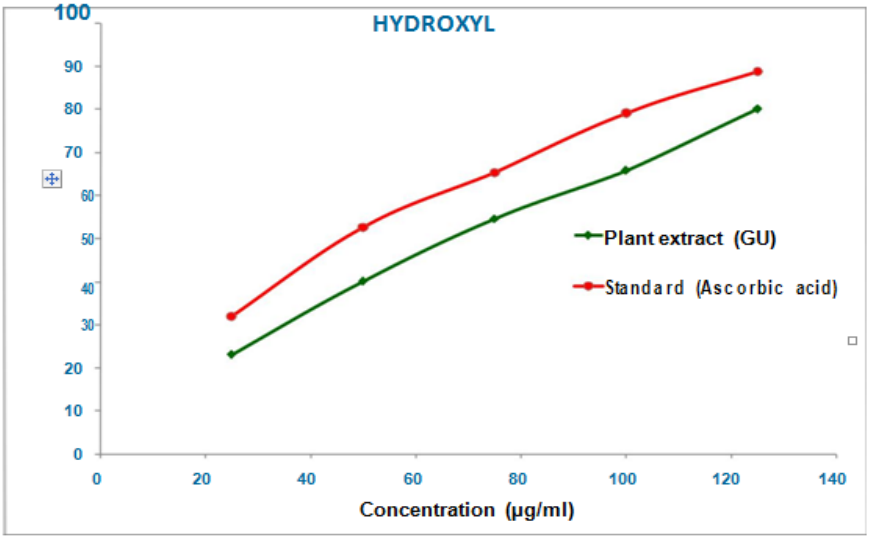


Fig 5.1 Graphical representation of Hydroxyl radical scavenging activity of ethanol extract of barks of *Grewia umbellifera*

f) Total Reducing Potential Assay

The percentage of inhibition in reducing potential in different concentration like 25, 50, 75, 100, 125 μg/ml were observed in 0.17±0.01, 0.25±0.02, 0.34±0.04, 0.59±0.03 and 0.75±0.02 respectively where as the percentage inhibition of ascorbic acid in concentration like 25, 50, 75, 100, 125 μg/ml were found to be 0.14±0.01, 0.20±0.01, 0.29±0.02, 0.41±0.02 and 0.62±0.03 respectively in Table 8 and Figure 6. The IC50 values for Total reducing potential activity of ethanol extract of barks of *Gmelina arborea* and ascorbic acid were 78 μg/ml and 80 μg/ml respectively. Values are the average of triplicate experiments and represented as mean ± standard deviation.

The percentage of inhibition in reducing potential in different concentration like 25, 50, 75, 100, 125 μg/ml were observed in 0.10±0.01, 0.12±0.01, 0.17±0.02, 0.28±0.03 and 0.40±0.01 respectively where as the percentage inhibition of ascorbic acid in concentration like 25, 50, 75, 100, 125 μg/ml were found to be 0.14±0.01, 0.20±0.01, 0.29±0.02, 0.41±0.02 and 0.62±0.03 respectively in Table 8.1 and Figure 6.1. The IC50 values for total reducing potential activity of ethanol extract of barks of *Grewia umbellifera* and ascorbic acid were 84 μg/ml and 80 μg/ml respectively. Values are the average of triplicate experiments and represented as mean ± standard deviation.

Table: 8 Total reducing potential activity ethanol extract of barks of *Gmelina arborea*

Group	**Concentration (μg/ml)**	**% of Inhibition**	**IC50 Value**
Ethanol extract of barks of *Gmelina arborea*	25	0.17±0.01	78 μg/ml
	50	0.25±0.02	
	75	0.34±0.04	
	100	0.59±0.03	
	125	0.75±0.02	
Ascorbic Acid	25	0.14±0.01	80 μg/ml
	50	0.20±0.01	
	75	0.29±0.02	
	100	0.41±0.02	
	125	0.62±0.03	

Table: 8.1 Total reducing potential activity ethanol extract of barks of *Grewia umbellifera*

Group	**Concentration (μg/ml)**	**% of Inhibition**	**IC50 Value**
Ethanol extract of barks of *Grewia umbellifera*	25	0.10±0.01	84 μg/ml
	50	0.12±0.01	
	75	0.17±0.02	
	100	0.28±0.03	
	125	0.40±0.01	
Ascorbic Acid	25	0.14±0.01	80 μg/ml
	50	0.20±0.01	
	75	0.29±0.02	
	100	0.41±0.02	
	125	0.62±0.03	

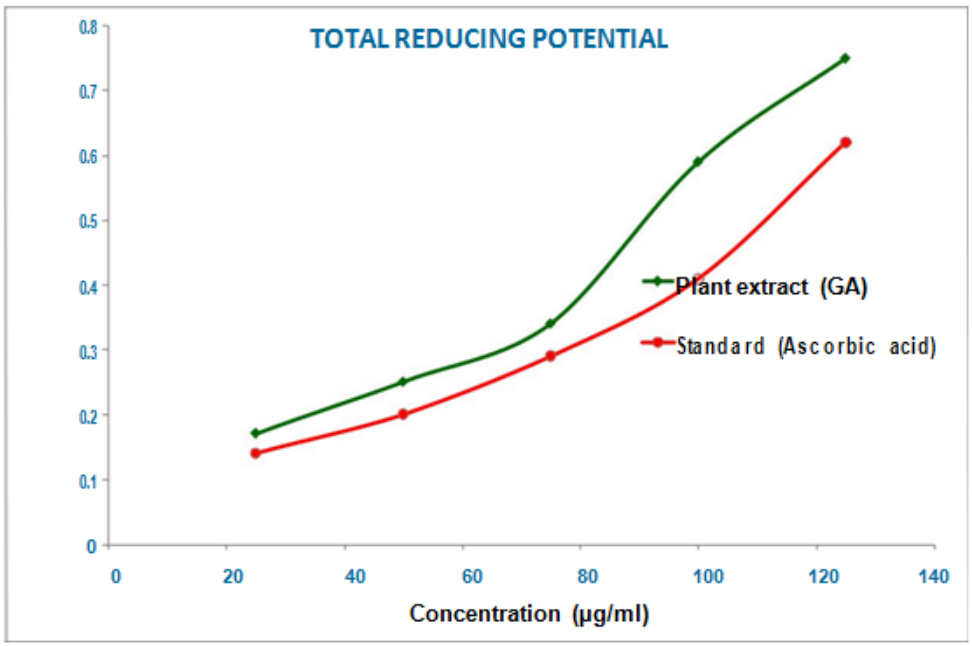


Fig 6 Graphical representation of Total reducing potential activity of ethanol extract of barks of *Gmelina arborea*

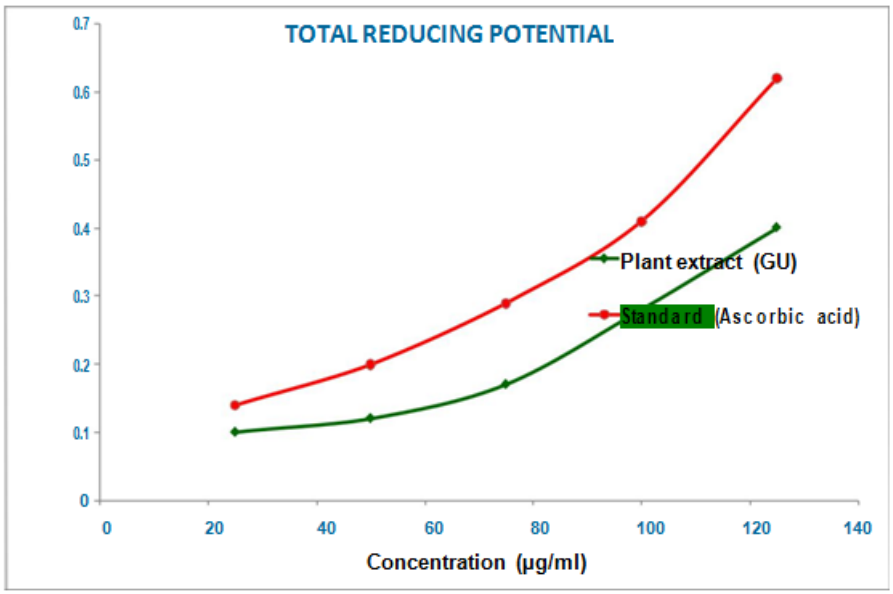


Fig 6.1 Graphical representation of Total reducing potential activity of ethanol extract of barks of *Grewia umbellifera*

g) Nitric Oxide Radical Scavenging Activity

Table 9 and Figure 7 depicts the percentage of inhibition of nitric oxide in different concentration like 25, 50, 75, 100, 125 µg/ml were observed in 43.11±1.69, 68.92±2.06, 81.75±1.77, 89.36±2.37 and 97.12±1.51 respectively where as the percentage inhibition of ascorbic acid in concentration like 25, 50, 75, 100, 125 µg/ml were found to be 32.18±1.82, 51.26±1.20, 68.89±2.09, 80.56±1.69 and 93.84±1.44 respectively. The IC50 values for nitric oxide radical scavenging activity for ethanol extract of barks of *Gmelina arborea* and ascorbic acid were 30 µg/ml and 44 µg/ml respectively. Values are the average of triplicate experiments and represented as mean

± standard deviation,Depicts the percentage of inhibition of nitric oxide in different concentration like 25, 50, 75, 100, 125 µg/ml were observed in 26.29±1.24, 39.53±1.98, 58.29±1.72, 73.66±1.37 and 86.12±1.59 respectively where as the percentage inhibition of ascorbic acid in concentration like 25, 50, 75, 100, 125 µg/ml were found to be 32.18±1.82, 51.26±1.20, 68.89±2.09, 80.56±1.69 and 93.84±1.44 respectively. The IC50 values for nitric oxide radical scavenging activity for ethanol extract of barks of

Grewia umbellifera and ascorbic acid were 55 µg/ml and 44 µg/ml respectively. Values are the average of triplicate experiments and represented as mean ± standard deviation.

Table: 9 NO radical scavenging activity of ethanol extract of barks of *Gmelina arborea*

Group	**Concentration (µg\ml)**	**% of Inhibition**	**IC50 Value**
Ethanol extract of barks of *Gmelina arborea*	25	43.11±1.69	30 µg/ml
	50	68.92±2.06	
	75	81.75±1.77	
	100	89.36±2.37	
	125	97.12±1.51	
Ascorbic Acid	25	32.18±1.82	44 µg/ml
	50	51.26±1.20	
	75	68.89±2.09	
	100	80.56±1.69	
	125	93.84±1.44	

Table 9.1 NO radical scavenging activity of ethanol extract of barks of *Grewia umbellifera*

Group	**Concentration (µg\ml)**	**% of Inhibition**	**IC50 Value**
Ethanol extract of barks of *Grewia umbellifera*	25	26.29±1.24	55 µg/ml
	50	39.53±1.98	
	75	58.29±1.72	
	100	73.66±1.37	
	125	86.12±1.59	
Ascorbic Acid	25	32.18±1.82	44 µg/ml
	50	51.26±1.20	
	75	68.89±2.09	
	100	80.56±1.69	
	125	93.84±1.44	

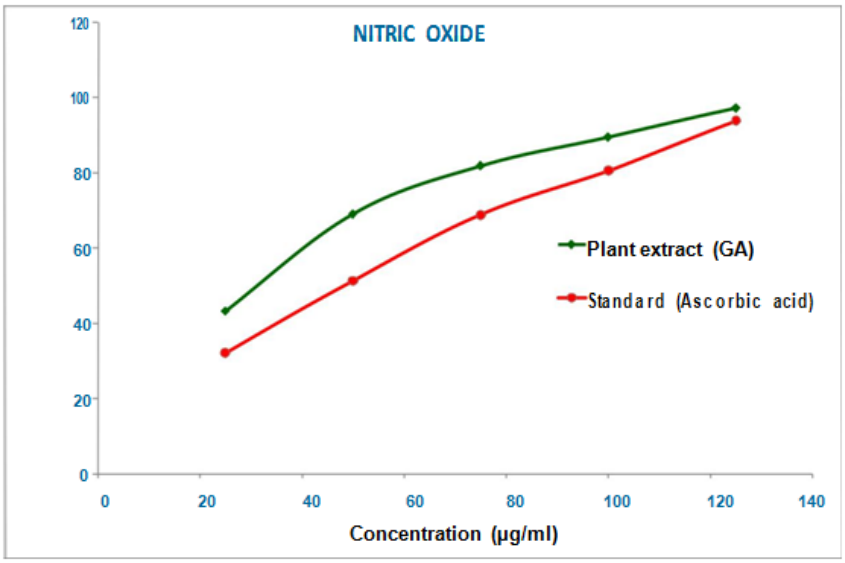


Fig 7 Graphical representation of NO radical scavenging activity of *Gmelina arborea*

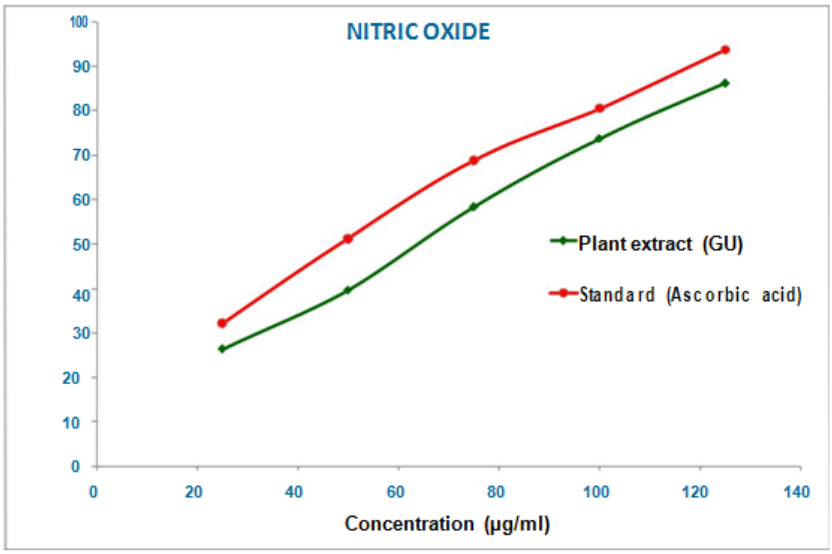


Fig 7.1 Graphical representation of NO radical scavenging activity of *Grewia umbellifera*

h) Acute toxicity of ethanol extract of barks of *GMELINA ARBOREA* AND *GREWIA UMBELLIFERA*

The results of the present study indicated that the toxicity of the ethanol extracts of barks of *GA* and *GU* were low. During the 14-days period of acute toxicity evaluation, no signs of toxicity were observed. Although the results showed significant differences in body weight gain, this suggested that the ethanol extracts of barks of *GA* and *GU* were of very low toxicity. The difference in the body weight slow by increased along the group. In group I (100 mg administered) the difference was which increased proportionately with the increase in the drug doses. In group V the difference in body weight before and after treatment was 9.58. During the duration of the treatment mortality was not reported in any other group other than group V. The percentage of mortality was 1 (Table. 5.21).

Table: 10 Acute toxicity effect of ethanol extract of GA and GU of different drug dosage (100, 200 and 300) and mortality rate in albino rats

Parameters	GA			GU		
	Group I 100 mg	Group II 200 mg	Group III 300 mg	Group IV 1000 mg	Group V 2000 mg	Group VI 3000 mg
Body Weight before treatment (g)	154.02±1.40	155.02±1.40	154.02±1.55	152.02±1.40	153.23±1.40	152.02±1.40
Body Weight after treatment (g)	150.33±0.60	152.00±1.30	151.69±1.53	149.81±2.20	149.44±4.20	142.44±4.20
Difference in body weight (g)	3.69	3.02	2.33	2.21	3.79	9.58
Mortality rate %	0	0	0	0	0	1

Values are expressed as mean ± SD for six rats in each group.

XVII. Conclusion

The present work is a comprehensive compilation of findings in the evaluation of traditionally known medicinal plants for their Hepatoprotective and nephroprotective activity of the two medicinal plants GA and GU. The medicinal plants GA and GU confirmed the presence of many phytoconstituents and among all the extract ethanol extract showed a significant number of phytochemicals in them and the ethanol extract were used for further studies.The spectral studies of the plants also revealed the presence of many components in them.The study on in vitro antioxidant activity of medicinal plants also revealed the antioxidant potential of the plants.Hepatoprotective activity studies of the barks of GA and GU on paracetamol induced rats showed the significant protective activity.The ethanol extract of GA and GU extracts showed significant nephroprotective activity. In conclusion, the results of the present investigation infer that these plants (GA and GU) extracts possess potent antioxidant, Hepatoprotective and nephroprotective property, the former being probably responsible than the later. Thus, the extracts can be beneficial in treating liver and renal damages caused due to chemical exposure.

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