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Membrane Stability and Antioxidant Activity of *Gmelina arborea* Seed Extracts and Their Fatty Acid Composition

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Authors' contributions

This work was carried out in collaboration by five authors. Author DK managed the literature searches, design the study, performed the statistical analysis wrote the protocol and first draft of the manuscript. Authors AS and RC managed the analyses of the study. Author SA managed the protocol of the study and author AKS managed the work done. All authors read and approved the final manuscript.

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ABSTRACT

Aims: In the present study different extracts of seeds of *Gmelina arborea* were prepared and evaluated their membrane stabilizing and antioxidant effects. The Fatty acid composition was also estimated.
Study Design: Fatty acid analysis by GCMS and evaluation of membrane stabilizing and antioxidant activity by hypotonic solution induced hemolysis and DPPH method respectively.
Place and Duration of Study: Department of Pharmaceutical Chemistry, Dolphin PG Institute of Biomedical and Natural Sciences, Dehradun, India, between October 2013 to September 2014.
Methodology: All extract were tested for presence of phytoconstituents i.e., alkaloid, carbohydrate, sterols, proteins, amino acids, saponin, and phenolic compounds in different extracts. Membrane Stabilizing effect was studied by hypotonic solution induced hemolysis of erythrocyte. Antioxidant activity was studied by DPPH method at a different concentration 50 µg/ml to 500 µg/ml. GCMS analysis was done for petroleum ether extract with the help of Perkin Elmer Clarus-500 model coupled with CLARUS-500 Mass spectrometer.
Results: Phytochemical analysis showed the presence of alkaloid, carbohydrate, sterols, proteins, amino acids, saponin, and phenolic compounds in different extracts. Methanol extract was the richest extract for tested phytoconstituents. Different fatty acids were present in petroleum ether extract which was analyzed by GCMS. Maximum membrane stabilizing activity of seeds of *Gmelina arborea* showed in acetone (45.39±0.84) and methanol (44.12±0.72) extracts at a concentration of 2000 µg/ml in comparison to standard drug. From antioxidant studies, methanol extract showed maximum antioxidant activity (91.99±0.46) at a concentration of 500 µg/ml than other extract in comparison to standard drug ascorbic acid.
Conclusion: From above studies it could be concluded that methanol and acetone extract showed maximum membrane stabilizing and antioxidant activities.

Keywords: Gmelina arborea; anti-inflammatory; antioxidant; DPPH; erythrocyte membrane stabilization; aspirin; ascorbic acid.

1. INTRODUCTION

The medicinal plants are widely used as a folklore medicine for the treatment of diseases all over the world. There is huge demand for the search for new anti-inflammatory and antioxidant agents from herbal plant. The inflammatory response is a critical protective reaction to irritation, injury, or infection, characterised by redness, heat, swelling, loss of function and pain [1]. Redness and heat results from an increase in blood flow, swelling is associated with increased vascular permeability, and pain is the consequence of activation and sensitisation of primary afferent nerve fibres [2]. Different inflammatory mediators are released at the time of tissue injury [3].

Secretion of phagocytes and release of O_2, OH radicals as well as non-free radical species (H_2O_2) are responsible for inflammation which can harm surrounding tissue either by causing oxidation or by hydrogen peroxide (H_2O_2) and .OH radicals formed from O_2, which initiates lipid peroxidation resulting in membrane destruction [4].

Cells use oxygen to form energy by breakdown proteins and fats. The human body uses its energy as a fuel. It also utilizes oxygen to help the immune system, destroys foreign substances and combats diseases. An antioxidant prevents oxidation of other molecules. Free radicals are the entities which damage cells. An antioxidants protect cells from damage caused by these free radicals. An antioxidants terminates all the reaction of free and inhibit other oxidation reactions by being oxidized themselves [5]**.** There are huge demand of search for novel natural antioxidants and anti-inflammatory agents of plant origin has ever since increased worldwide. Plant derived drugs are safe and have no side effects. It is not known which constituents of plant are associated in reducing the risk of chronic diseases, but they appear to play a major role in the protective effect of plant medicine. *Gmelina arborea* belongs to belongs to the family Verbenaceae. It is khown as Gamhar in Hindi and White teak in English and is widely destributed throughout in South Asia, Pakistan, Bangladesh, China, Japan, Myanmar, Nepal, Pakistan, Sri Lanka, Thailand and India [6,7]. The leaf of *Gmelina arborea* possesses anthelmintic [8], antioxidant and Cardioprotective

[9], ameliorating properties [10], antiulcer [11], analgesic [12], antimicrobial [13] and antihyperlipidemic activities [14]. The bark and stem bark possesses antidiabetic [15], antipyretic [16], analgesic [16], anti-inflammatory [17], antioxidant [18], ameliorating activities [10]. Furthermore root of *Gmelina arborea* showed immunomodulatory activity [19], antipyretic [20], anthelmintic activities [21].

Very few research work available on the seed of *Gmelina arborea specially* in the control of inflammation and antioxidant effect. This study therefore, reports the anti-inflammatory or membrane stabilizing properties and antioxidant effect of seed extract of *Gmelina arborea* as well as the fatty acid analysis.

2. EXPERIMENTAL DETAILS/METHODOLOGY

2.1 Collection & Identification of Seeds of *Gmelina arborea*

Seeds of *Gmelina arborea* were collected from Dehradun (India). Plant material was authenticated by Dr. Manisha Thapliyal (Scientist-D & officer incharge Forest Tree Seed Laboratory), in Silviculture Division, Forest Research Institute, Dehradun, India. DPPH, aspirin and ascorbic acid were purchased from HIMIDEA, Mumbai, India.

2.2 Extraction of Seeds of *Gmelina arborea* in Different Solvents

The dried seeds (1000 gm.) of *Gmelina arborea* were crushed. The crushed Seeds extracted with different solvents of increasing polarity viz. Petroleum ether, Chloroform, Acetone and Methanol by cold percolation method. The extract was evaporated till dryness to obtain residue. These extracts were concentrated under reduced pressure [22].

2.3 Phytochemical Analysis

All extract were tested for presence of phytoconstituents i.e., alkaloid (mayer's test), carbohydrate (molish test), sterols (salkowaski test), proteins, amino acids (Ninhydrin test), saponin Foam test, and phenolic compounds (ferric chloride test) in different extracts. [22].

2.4 GC-MS (Gas Chromatography Mass Spectroscopy) Analysis of Petroleum Ether Extract (oil) of *Gmelina arborea* Seed

The extracted petroleum ether extract (oil) was subjected to GC-MS analysis. The GC-MS analysis of oil was carried out on a Shimadzu Mass spectrometer (GCMS Solutions). Equipment: GC Clarus 500 Perkin Elmer. Injection volume was 0.1 ul in the (split flow 50ml/minute). Helium as a carrier gas at a flow rate of 1ml/min. Detector: Mass detector Turbo mass gold- Perkin Elmer, Software: Turbo mass. Mass spectral identification were made by matching the mass against the NIST library software and the retentional time comparison with the publisher data of Wiley dated 28/08/14 [23].

2.5 Antioxidant Activity of Seed Extracts

2.5.1 DPPH method

Weigh accurately 20 mg DPPH and dissolved in 100 ml methanol. Generally Methanol and for some cases Ethanol is used as a solvent for DPPH. Standard solution of ascorbic acid is prepared. *viz. 50, 100, 200, 300, 400, and 500 µg/ml respectively.* Different concentration of the test samples of *Gmelina arborea* extracts which is to be examined for anti-oxidant activity is prepared in their respective solvent *viz. 50, 100, 200, 300, 400 and 500, µg/ml.* For analysis of test samples, 3 ml of different concentration of test sample *Gmelina arborea* extract was mixed with 1 ml of DPPH solution in dark. For analysis of Standard drug Ascorbic acid, 3 ml of different concentration of standard solution of ascorbic acid was mixed with 1 ml of DPPH solution in dark. The prepared solution of ascorbic acid and test samples was incubated for 1/2 half an hour. Procedure is done, absorbance is taken with the help of U.V. Spectrophotometer at 517 nm. Calculate the % activity of individual concentration of individual extract from the following formula:- [24,25]

$$\%\ Activity = \frac{Abs.of\ control - Abs.\ of\ individual\ concentration}{Abs.of\ control} \times 100$$

Abs. = Absorbance.

2.6 Membrane Stabilization Activity of Seed Extracts

2.6.1 Effect on hemolysis

2.6.1.1 Erythrocyte suspension

Whole blood was collected from goat from slaughter house and NIH-ACD (National Institute of Health-Acid Citrate Dextrose) solution was added to it to prevent clotting. The blood was centrifuged three times with 0.9% saline. The volume of saline was measured and reconstituted as a 40% (v/v) suspension with isotonic buffer solution (pH 7.4). Which contained in 100 ml of distilled water: $NaH_2PO_4.2H_2O$, 0.26 g; Na_2HPO_4, 1.15 g; NaCl, 9 g (10 mm sodium phosphate buffer). The isotonic buffer solution was composed of 154 mm NaCl in 10 mm sodium phosphate buffer (pH 7.4).

2.6.1.2 Hypotonic solution-induced hemolysis

Stock erythrocyte suspension (30 µl) was mixed with 5 ml of the hypotonic solution containing the Gmelina arborea seed extracts at concentrations of 1000, 1500 and 2000, µg/ml, while the control sample was mixed with drug free solution. The mixtures were incubated for 10 min at room temperature, and centrifuged at 3000 g for 10 min. All the experiments were performed in triplicates and the absorbance (O.D.) of the supernatant was measured at 560 nm. Aspirin was used as a reference standard [26-29].

2.6.1.3 Calculation and statistical analysis

The percentage inhibition or acceleration of hemolysis in tests (b) and (c) was calculated according to the equation:

$$\% \text{ acceleration or inhibition of hemolysis} = 100x \left[\frac{OD1-OD2}{OD1}\right]$$

Where,

OD_1 = Optical density of hypotonic saline solution + blood (control) and

OD_2 = Optical density of test sample in hypotonic saline solution + blood.

Results are expressed as percentage mean values ± standard error (n = 3)

3. RESULTS AND DISCUSSION

The curshed seeds are extracted by different solvents i.e. petroleum ether, chloroform, acetone, methanol by cold percolations and the yield of seeds extracts in different solvent systems are Pet.ether (51 ml), Chloroform (5.018 gm), and Acetone (2.012 gm) Methanol (3.254 gm).

For the pharmacological discovery of novel drugs, the primary essential information regarding the chemical constituents are generally provided by the qualitative phytochemical screening of plant extracts [30]. The extracts of seed of *Gmelina arborea* was tested for various qualitative chemical tests. From the result we found out that methanol extract was the richest extract for phytoconstituents. It contains all tested phytoconstituents viz. Alkaloids. Carbohydrate compounds, Phenolic compounds and tannins, proteins and amino acid, fats and fixed oil. Acetone extract contain alkaloid, carbohydrates, Phenolic compounds and tannins, proteins and amino acid. Chloroform extract and Petroleum ether extract both does not contain any phytoconstituents.

3.1 GCMS Analysis of Petroleum Ether Extract

GCMS analysis of petroleum ether extract contains n-hexadecanoic acid (8.50%), Oleic acid (73.27%), 9-Octadecanoic acid (6.06%), 9,12-Octadecadienoic acid- Linoleic acid (0.10%) (Fig. 1).

3.2 *In vitro* Anti-inflammatory Activity

The anti-inflammatory activity of the different extracts were compared with standard drug Aspirin at 560 nm. It was observed that the concentration of 2000 µg/ml of acetone extract showed maximum activity (45.39%). Methanol extract showed 44.12% activity at a concentration of 2000 µg/ml. Petroleum ether and chloroform extract showed very less effect on hemolysis in comparision to other exract and aspirin (Table 1).

Inflammation mediated release of lysosomal constituents which cause inflammation and damage of cells. Stabilization of lysosomal cell membrane inhibits the release of lysosomal constituents from it [31]. Erythrocyte membrane is same as lysosomal membrane, and by stabilize the erythrocyte membrane with extracts may also stabilize lysosomal membrane [32]. Stabilization of erythrocyte cell membrane by hypotonic solution induced erythrocyte membrane lysis can be taken as an in vitro

measure of anti-inflammatory activity of the drugs or plant extracts.

3.3 Antioxidant Activity

Methanol extract of *seeds of Gmelina arborea* showed maximum antioxidant activity in comparison to all extracts. The concentration of 500 µg/ml of methanol extract showed 91.99% anti-oxidant activity in comparison to all extract and standard drug. At a concentraton of 500 µg/ml of petroleum ether extract showed less antioxidant activity 67.54% where as chloroform extract and acetone extract showed 75.0% and 86.63% antioxidant activity (Table 2). DPPH scavenging method is based on the addition of radical species and antioxidants which scavenges by DPPH and there is change in colour of DPPH solution. The change of colour of DPPH solution is depend on the concentration and potency of antioxidants. Decrease in absorbance of reaction mixture indicates significantly the antioxidant activity [33]. The other parts of *Gmelina arborea* plant have also reported the significant anti-inflammatory and antioxidant activities. Seed of *Gmelina arborea* also showed significant anti-inflammatory and antioxidant as compare with other plant parts [9, 14,17,18]. In the present study methanol and acetone extracts showed significantly higher inhibition percentage. Results of this study suggest that the plant extract contain phytochemical constituents that are capable of donating hydrogen to a free radical to scavenge the potential damage [34].

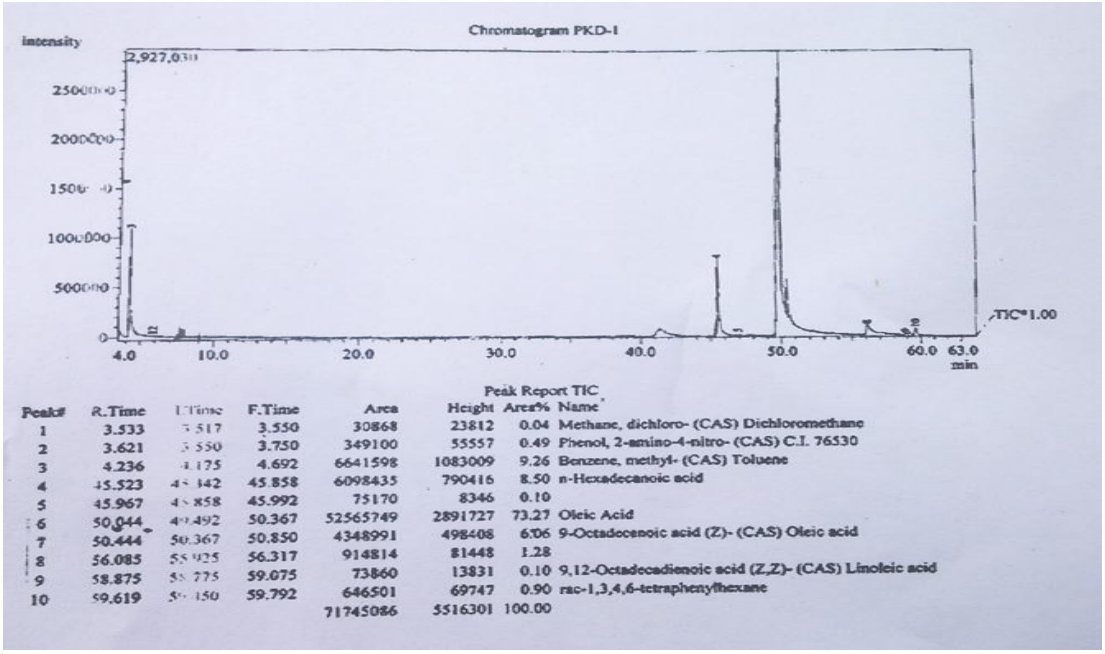

Fig. 1. GCMS spectra of petroleum ether extract

Table 1. % inhibition of different extracts on hemolysis

S. no.	Concentration (µg/ml)	% Inhibition of hemolysis of extracts				Standard drug	Standard drug at concentration (µg/ml)
		Pet. ether	Chloroform	Acetone	Methanol	Aspirin	
1	Control	-	-	-	-	-	
2	1000	8.83±0.22	14.59±1.32	18.16±0.70	29.66±0.61	48.80±0.47	100
3	1500	14.24±1.07	14.74±1.16	28.36±0.67	34.84±1.51	56.85±1.15	150
4	2000	14.78±0.56	16.97±0.21	45.39±0.84	44.12±0.72	58.88±1.0	200

**Results are expressed as percentage mean values ± standard error (n = 3)*

Table 2. % antioxidant activity of different extracts

S. no.	Concentration (µg/ml)	% antioxidant acitivity				Standard drug
		Pet. ether	Chloroform	Acetone	Methanol	Ascorbic acid
1	Control	-	-	-	-	-
2	50	23.43±0.73	17.99±0.67	32.38±0.58	47.21±0.20	96.45±0.13
3	100	52.12±0.91	62.47±0.98	44.48±0.72	64.72±0.74	96.62±0.22
4	200	57.43±0.66	62.92±0.57	63.58±0.90	78.16±0.46	96.31±0.15
5	300	59.18±0.48	65.01±0.43	70.89±0.30	90.50±0.68	96.47±0.16
6	400	62.12±0.87	67.47±0.43	82.73±0.97	91.81±0.33	96.34±0.33
7	500	67.54±1.36	75.00±0.78	86.63±0.62	91.99±0.46	96.38±0.20

Results are expressed as mean values ± standard error (n = 3)

4. CONCLUSION

From the above study it is concluded that methanol extract of plant showed the increase in the protection of the erythrocyte membrane against hypotonic haemolysis and less protection shown by Acetone, chloroform and Petroleum ether.

From antioxidant activity of different plant extracts of *Gmelina arborea,* maximum activity showed by methanol extract of *Gmelina arborea* seed comparison with standard drug ascorbic acid at 517 nm, except other extract showed less activity as compare to methanol extract. Further study needed for the isolation of active principle.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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