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Membrane Stabilizing and Antioxidant Activity of *Ougeinia oojeinensis* Seed Extracts and Their Fatty Acid Composition.

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ABSTRACT

In the present study different extracts of seeds of *Ougeinia oojeinensis* were prepared and evaluated their membrane stabilizing and antioxidant effects. The Fatty acid composition was also estimated. 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 haemolysis of erythrocyte. Antioxidant activity was studied by DPPH method at a different concentration. GCMS analysis was done for petroleum ether extract with the help of Perkin Elmer Clarus-500 model coupled with CLARUS-500 Mass spectrometer. Phytochemical analysis showed that methanol extract was the richest extract for the tested phytoconstituents. Methanol extract showed the presence of alkaloid, carbohydrate, saponin, protein, amino acids and phenolic compounds. Different fatty acids were present in petroleum ether extract which was analyzed by GCMS. Maximum membrane stabilizing activity of seeds of *Ougeinia oojeinensis* showed in Methanol extract (81.41±1.28) at a concentration of 1000 µg/ml in comparison to standard drug aspirin. From antioxidant studies, methanol extract showed maximum antioxidant activity (91.31±1.31) at a concentration of 1000 µg/ml than other extract in comparison to standard drug ascorbic acid. From above studies it could be concluded that methanol extract showed maximum membrane stabilizing and antioxidant activities.

Keywords: *Ougeinia oojeinensis*; anti-inflammatory; antioxidant; DPPH; erythrocyte membrane stabilization; aspirin; ascorbic acid.

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INTRODUCTION

From thousands of years plants have been used as a traditional medicine system for the treatment of various diseases for human being throughout the world. Herbal drugs are more important as compared to other modern medicine because of low cost, easy availability and non-toxic in nature. Medicinal plants are best source of medicine for the treatment of various diseases [1]. Inflammation can be defined as a defensive but exaggerated local tissues reaction in response to exogenous or endogenous insult. It is a complex phenomenon, comprising of biochemical as well as immunological factors. It is recognised by calor (heat), rubor (redness), tumor (swelling) and dolor (pain). Tissue damage initiates or activates chemotactic factors that provoke directly or indirectly the appearance of the mediators of pain and inflammation [2]**.** The inhibition of COX-2 and prostaglandin synthesis are main function and mode of action of NSAIDs due to this there is some toxic adverse effects appear like gastric mucosal damage, asthma, bleeding, inhibition of platelet function and anaphylactic reactions [3]. Free radicals are very harmful to the human body. They are capable of cell damage and appears to be major contributor to degenerative diseases [4].

The harmful effects of free radicals are counteract by different enzymatic and non-enzymatic antioxidant defences in the human body. There are large number of diseases including cancer[5], cardiovascular disease[6], neural disorders[7], Alzheimer's disease[8], mild cognitive impairment[9], Parkinson's disease[10], alcohol induced liver disease[11], ulcerative colitis[12], aging[13] and atherosclerosis[14] caused by free radicals.

The dietary intake of antioxidants may protect against free radicals. Research indicates that antioxidant rich foods and nutrients play a major role in prevention of disease. Therefore the combination of antioxidants may be more effective over the long term. Antioxidants also more important in prevention of degenerative diseases and improving the quality of life.

Ougeinia oojeinensis (Roxb.) Hochr belongs to Fabaceae family and known Tinsa in Hindi, Ratha in Sanskrit found in outer Himalayas and sub-Himalayan tracts from Jammu to Bhutan [15,16]. It is deciduous medium sized herb and shows potential antibacterial, antioxidant and anti-cancer activity[17], hypoglycaemic activity[18], antidepressant activity[19]. *Ougeinia oojeinensis* have reported the presence of phytoconstituents lupeol, hydroxlupeol, betulin and isoflavanones such as dalbergioidin, homoferreirin and ougenin [20-22].

MATERIAL AND METHODS

Collection & Identification of Seeds of *Ougeinia oojeinensis*

Seeds of *Ougeinia oojeinensis* were collected from Dehradun (India). Seed materials 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.

Extraction of Seeds of *Ougeinia oojeinensis* in different Solvents

The dried seeds (1000 gm.) of *Ougeinia oojeinensis* were crushed. The crushed Seeds extracted with different solvents of increasing polarity viz. Petroleum ether, Acetone and Methanol by hot percolation method. The extract was evaporated till dryness to obtain residue. These extracts were concentrated under reduced pressure [23].

Phytochemical analysis

All extracts were tested for presence of phytoconstituents i.e., alkaloid, carbohydrate, sterols, proteins, amino acids, saponin test, and phenolic compounds [23].

GC-MS (Gas Chromatography Mass Spectroscopy) Analysis of Petroleum Ether Extract (oil) of *Ougeinia oojeinensis* Seed

The 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 was made by matching the mass against the NIST library software and the retention time comparison with the publisher data of Wiley [24].

Membrane Stabilizing Activity of Seed Extracts

Effect on haemolysis

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).

Hypotonic solution-induced hemolysis

Stock erythrocyte suspension (30 µl) was mixed with 5 ml of the hypotonic solution containing the *Ougeinia oojeinensis* seed extracts at concentrations of 500 and 1000 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 [25-28].

Calculation and statistical analysis

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

$$\% \text{ inhibition of haemolysis} = \frac{OD_1 - OD_2}{OD_1} \times 100$$

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)

Antioxidant Activity of Seed Extracts

DPPH method

Weigh accurately 20 mg DPPH and dissolved in 100 ml methanol. Standard solution of ascorbic acid is prepared as 100 µg/ml respectively. Different concentration of the test samples of *Ougeinia oojeinensis* extracts which is to be examined for anti-oxidant activity is prepared in their respective solvent viz. 250, 500 and 1000 µg/ml. For the analysis of test samples, 3 ml of different concentration of test sample of *Ougeinia oojeinensis* extracts were 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 30 minutes. When Procedure is done; absorbance is taken with the help of U.V. Spectrophotometer at 517 nm.

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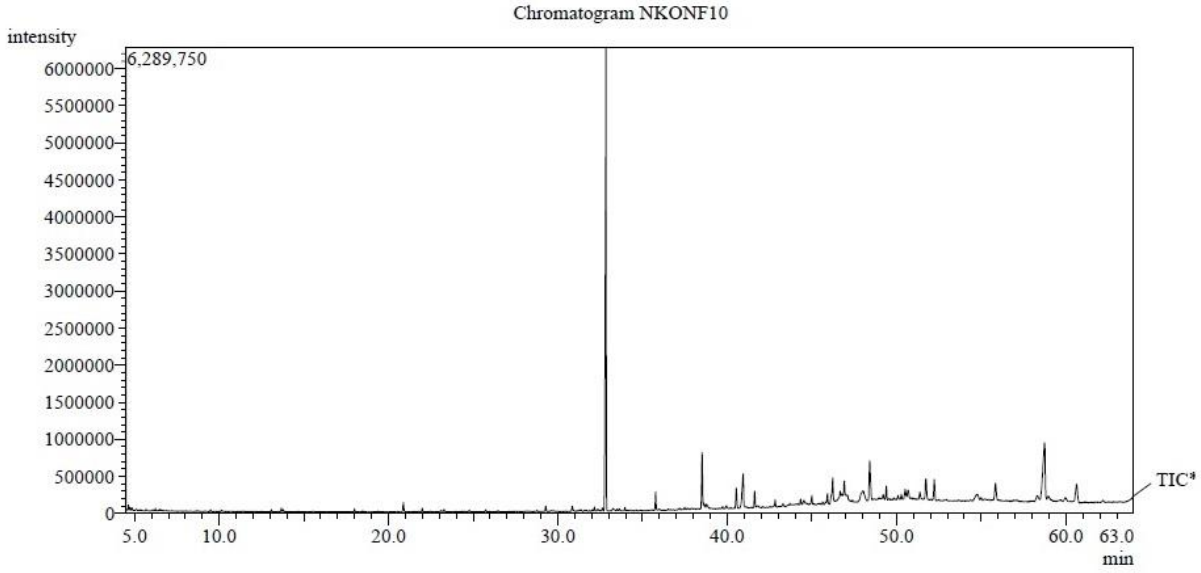
Calculate the % activity of individual concentration of individual extract from the following formula:- [29-30]

$$\% \text{ Antioxidant Activity} = \frac{\text{Absorbance of Control} - \text{Absorbance of Individual Concentration}}{\text{Absorbance of Control}} \times 100$$

RESULTS AND DISCUSSION



Peak Report TIC

Peak#	R.Time	Area	Height	Area%	Name
1	32.836	23605703	6248566	52.38	3-Methyl-4,6-di-tert-butyl-phenol
2	35.775	771537	243866	1.71	pentadecane
3	38.515	2481518	728421	5.51	Sulfurous acid, cyclohexylmethyl hexyl ester
4	40.536	925434	255569	2.05	4-Hydroxy-3,5-di-tert-butylbenzaldehyde
5	40.940	1905999	406900	4.23	Tetradecanoic acid
6	41.615	731807	217027	1.62	Tetracosane (CAS) n-Tetracosane
7	45.918	406704	124842	0.90	Decane, 5,6-bis(2,2-dimethylpropylidene)-, (E,Z)-
8	46.237	898516	265044	1.99	Eicosanoic acid
9	46.916	687911	195455	1.53	Hexatriacontane (CAS) n-Hexatriacontane
10	48.416	2733391	523652	6.07	PROPAN, 1-(DI-TERT.BUTYLPHOSPHINO)-3-(2,4,6-TRI-TERT.BUTYLPHENYL)PHO
11	49.397	499831	159322	1.11	2-methyltetracosane
12	51.738	1175856	251338	2.61	Tetradecanamide
13	52.230	1190220	269077	2.64	2-methyltetracosane
14	55.842	991052	190074	2.20	2-methyltetracosane
15	58.735	6061076	719212	13.45	9-Octadecenamide, (Z)- (CAS) OLEOAMIDE
		45066555	10798365	100.00	

Figure 1: GCMS spectra of Petroleum ether extract of seed of *Ougeinia oojeinensis*

DISCUSSION

The cursed seed are extracted by different solvents i.e. petroleum ether, acetone and methanol by hot percolations method and the yield of seeds extracts in different solvent systems are Petroleum ether extract (72 ml), Acetone extract (1.520 gm) and Methanol extract (2.350 gm).

The extracts of seed of *Ougeinia oojeinensis* undergo various qualitative phytochemical tests. They showed their presence and absence in the different solvent systems. From the results, we found out that

methanol extract was the richest extract for phytoconstituents except sterols. It contains all tested phytoconstituents viz. Alkaloids, carbohydrates, Phenolic compounds, saponins and proteins and amino acids. Acetone extract showed presence of alkaloids, carbohydrates and phenolic compounds only. Petroleum ether extract showed only presence of steroid.

GCMS Analysis of Petroleum Ether Extract

GCMS analysis of petroleum ether extract contains Tetradecanoic acid (4.23 %), Eicosanoic acid (1.99 %) and other compounds.

Membrane Stability Activity:

The membrane stability activity of the different extracts was compared with activity of standard drug aspirin at 560 nm. It was observed that the concentration of 1000 µg/ml of methanol extract showed maximum activity of 81.41 % in comparison with other extract and standard drug aspirin.

Inflammation may be defined as the series of changes that occurs in living tissues following injury. The injury which is responsible for inflammation may be brought about by a variety of conditions such as physical agents like mechanical trauma, ultra-violet or ionizing radiation; chemical agents like organic and inorganic compounds, the toxins of various bacteria; intracellular replication of viruses; hypersensitivity reactions like reaction due to sensitized lymphocytes with antigenic material viz., inhaled organic dusts or invasive bacteria; and necrosis of tissues whereby inflammation is induced in the surrounding tissues [31].

The release of lysosomal constituents by inflammation mediated response causes inflammation and damage of cell. Stabilization of lysosomal membrane inhibits the release of lysosomal constituents results in reduction of inflammation [32]. RBC membrane is resemblance to lysosomal membrane and by stabilize RBC membrane may also stabilize lysosomal membrane [33]. Stabilization of RBC membrane by hypotonic solution induced RBC membrane lysis can be taken as an in vitro measure of anti-inflammatory activity of the drugs or plant extracts.

Antioxidant activity

Methanol extract of seeds of *Ougeinia oojeinensis* showed maximum antioxidant activity in comparison to all extracts and standard drug ascorbic acid. The concentration of 1000 µg/ml of methanol extract showed 91.31 % antioxidant activity. The DPPH 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 colour change of DPPH solution depends on the concentration and potency of antioxidants. Decrease in absorbance of reaction mixture indicates significantly the antioxidant activity [34].

Table 1: Effect of different extract and standard drug on membrane stabilizing activity:

Concentration of extracts (µg/ml)	% membrane stabilizing activity of extracts & Standard Drug				
	Ougeinia oojeinensis seed extracts			Standard Drug	
	Petroleum ether	Acetone	Methanol	Acetyl Salicylic acid	Concentration of Acetyl Salicylic acid (µg/ml)
500	11.67±1.03	60.79±0.58	49.41±1.72	49.14±0.77	**100**
1000	18.71±0.72	71.27±1.42	81.41±1.28	55.66±0.75	**150**

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

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Table 2: Effect of different extract and standard drug on antioxidant activity:

Concentration of extracts (µg/ml)	% antioxidant activity of extracts & Standard Drug				
	Ougeinia oojeinensis seed extracts			Standard Drug	
	Petroleum ether	Acetone	Methanol	Ascorbic Acid	Concentration of Ascorbic Acid (µg/ml)
250	-	28.26±1.07	63.05±1.09	96.50±0.19	**100**
500	-	29.31±0.77	88.79±1.15		
1000	-	33.55±0.63	91.31±1.31		

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

CONCLUSION

From the above study it is concluded that methanol extract of *Ougeinia oojeinensis* seed showed remarkable membrane stabilizing activity and antioxidant activities. Further study needed for the isolation of active principle.

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