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PRELIMINARY PHYTOCHEMICAL, *IN VITRO* ANTIOXIDANT AND ANTIINFLAMMATORY ACTIVITY OF DIFFERENT EXTRACTS OF *ELEPHANTOPUS SCABER* LINN.

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ABSTRACT

The present study was aimed to evaluate the phytochemical and antioxidant and anti-inflammatory nature of the different extracts of aerial parts of *Elephantopus scaber* plant. Initially, the plant material was collected authenticated and dried for powdering. The dried material was powdered in mechanical grinder and the coarse powder thus obtained was extracted in soxhlet apparatus with the solvents of increasing polarity viz., petroleum ether, chloroform, ethyl acetate and methanol. The dried extracts thus obtained were subjected to preliminary phytochemical evaluation. *In vitro* antioxidant activity of all the extracts was evaluated by DPPH, reducing power assay, superoxide, nitric oxide and hydroxyl free radical scavenging assay methods and the *in vitro* anti-inflammatory was evaluated by inhibition of protein denaturation, proteinase inhibitory activity and human red blood cell (HRBC) membrane stabilization assay. The results of preliminary phytochemical evaluation revealed the presence of glycosides, phenolic compounds, flavonoids, terpenoids, carbohydrates, protein, sterol and saponins in the tested extracts. The methanol and ethyl acetate extracts showed a significant presence of majority of phytochemicals comparing with other two, the petroleum ether, chloroform extracts. The methanol extract showed significant activity in the antioxidant and anti-inflammatory evaluation. These results are useful for further investigation in the future.

KEYWORDS: *Elephantopus scaber*, Preliminary phytochemical evaluation, *In vitro* antioxidant activity, *In vitro* anti-inflammatory activity.

INTRODUCTION

The utilization of plants as a source of medicinal remedies for disease treatment dates back to prehistoric eras.[1] India boasts one of the most ancient, affluent, and varied cultural heritages linked to the application of medicinal plants.[2] Traditional medicine derived from plants encompasses a broad spectrum of compounds that can be employed in the treatment of both chronic and infectious diseases.[3]

In recent times, there has been a growing focus on traditional herbal medicines within global health discussions. From 1983 to 1994, 78% of the new drugs sanctioned by the FDA were either derived from unaltered natural products or were semi-synthetic drugs obtained from natural sources.[4] There is widespread optimism that research into traditional herbal medicine will significantly contribute to global health initiatives. Additionally, the industry has allocated millions of US dollars in the pursuit of promising medicinal herbs and innovative chemical compounds.[5]

A diverse array of plant parts, including roots, stems, flowers, fruits, twigs, exudates, and modified organs, has been utilized for the extraction of raw medicinal substances. The therapeutic properties of these plants are attributed to specific chemical compounds that elicit distinct physiological effects on the human body. These compounds, known as phytochemicals, encompass alkaloids, glycosides, saponins, resins, oleoresins, sesquiterpenes, lactones, and both essential and fixed oils.[1]

The increasing demand for plant-derived pharmaceuticals necessitates a thorough assessment of plants utilized in traditional medicine for a range of health issues. Consequently, it is essential to investigate medicinal plants for their potential biological effects.[6] In light of this, the current study focused on analyzing the phytochemical properties and antioxidant and anti-inflammatory efficacy of extracts of the plant *Elephantopus scaber*, aiming to establish a foundation for future research.

MATERIALS AND METHODS

Plant collection and identification

The aerial parts of *Elephantopus scaber* was collected from the banks of Vellayani Lake located in the Thiruvananthapuram District of Kerala. Identification and authentication of collected plant material was done by Dr. S. Sukumaran, Associate Professor, Dept. of Botany & Research Centre and Dr. G. Johnsi Christobel, Head., Dept. of Botany & Research Centre, Nesamony Memorial Christian College, Marthandam, Kanyakumari District-629 165, Tamil Nadu.

Preparation of powdered material and extraction

Powdering and extraction of collected plant material was done in reference with the standard procedure.[6-11] The aerial part of the collected plant was in shade for about three weeks, powdered the dried material by using mechanical grinder and stored in the airtight container for further researches. Initially, the powdered material was extracted with the solvents of increasing polarity viz., petroleum ether, chloroform, ethyl acetate and methanol in the soxhlet apparatus assembly. For that, about 50g of dried coarse powder was weighed, moistened with the selected solvents, packed in the extracting apparatus and extracted with 500ml of each solvent individually. After each extraction, subsequent extraction was done by using the same dried marc. Each extract was filtered, distilled off the solvent to obtain the dried extract. The percentage yield of each dried extracts obtained was calculated.

Preliminary phytochemical screening

The preliminary phytochemical screening (test for alkaloids, glycosides, phenolic compounds and tannins, flavonones and flavonoids, carbohydrates, proteins and aminoacids, terpenoids, saponins, gum and mucilage and volatile oil) of the collected extracts were carried out in reference with the standard procedure.[6-10]

In vitro antioxidant activity

In vitro antioxidant evaluation of all the prepared extracts of *E. scaber* was evaluated by different approaches such as DPPH, reducing power assay, superoxide, nitric oxide and hydroxyl free radical scavenging assay methods in reference with the standard procedure.[10,12,13]

DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay

Test extracts (1ml) in different concentration (50, 100, 150 and 200µg/ml) was mixed with methanolic solution of DPPH (1ml; 0.1mM). This reaction mixture was incubated at room temperature in dark condition for 30min. After that, the absorbance of reaction mixtures was measured spectrophotometrically (517nm). Methanol was used as blank; DPPH in methanol was employed as control and the ascorbic acid as standard control. The percentage inhibition of DPPH radical by the *Crotalaria biflora* extracts was determined by

$$\text{Percentage inhibition} = \frac{\text{Control Abs.} - \text{Sample Abs.}}{\text{Control Abs.}} \text{ X } 100$$

Percentage inhibition Vs. Concentration was plotted as a graph. The IC_{50} values of the tests were assessed from the regression equation of the graph.

Reducing power assay

Test extracts (500µl) in different concentration (50, 100, 150 and 200µg/ml) was mixed with sodium phosphate buffer (1.5ml; 0.2M; pH 6.6) and potassium ferricyanide (1.5ml; 1%) This mixture was kept for incubation (50°C; 20min). Then, trichloroacetic acid (5ml; 10%) was added to the incubated mixture and subjected to centrifugation (3000rpm; 6min; 4°C). Upper layer of the centrifuged was collected. Collected upper layer solution (1.5ml) was mixed with equal volume of distilled water and fresh ferric chloride solution (300µl; 0.1%) and kept for 10min. Then, the absorbance of reaction mixture was measured spectrophotometrically (700nm). Ascorbic acid was employed as standard control for comparative evaluation.

Superoxide radical scavenging assay

The reaction mixture (3ml) contains test extracts in different concentration (50, 100, 150 and 200µg/ml), Nitro blue tetrazolium (0.1ml; 1.5mM) solution, EDTA (0.2ml; 0.1M), riboflavin (0.05ml; 0.12mM) and phosphate buffer (2.55ml; 0.067M). Control tubes were prepared wherein DMSO was added instead of sample. The reaction mixture was kept (30 min) in front of fluorescent light (34 W) and then the absorbance was measured spectrophotometrically (560nm). Ascorbic acid was used as the standard control. All the tests were performed in triplicate and the results were averaged. The percentage inhibition superoxide radical was calculated by

$$\% \text{ inhibition} = \frac{\text{Control Abs.} - \text{Sample Abs.}}{\text{Control Abs.}} \text{ X } 100$$

The IC_{50} indicated the concentration of the tests that inhibited 50% of radical.

Nitric oxide radical scavenging assay

1ml of Griess reagent (1% sulphanilamide in 5% phosphoric acid and 0.1% naphthyl ethylene diamine dihydro chloride) was added to the reaction mixture (5ml) which was prepared by mixing different concentration of test extracts (50, 100, 150 and 200µg/ml) with sodium nitroprusside (5mM) in phosphate buffer (pH 7.3). This reaction mixture was kept in 25°C (in front of 25W tungsten lamp) for 3h. The nitric oxide radical thus formed was interacted with oxygen to produce nitrite ion which was measured spectrophotometrically (546nm). Normal and standard control (Ascorbic acid) were prepared. The percentage inhibition of nitric oxide radical formation was determined by

$$\% \text{ inhibition} = \frac{\text{Control Abs.} - \text{Sample Abs.}}{\text{Control Abs.}} \text{ X } 100$$

Hydroxyl radical scavenging assay

Test extracts (100µl) in different concentration (50, 100, 150 and 200µg/ml) was mixed with deoxyribose (0.1ml; 2.8mM) prepared in potassium phosphate buffer (20mM; pH 7.4), EDTA (0.1mM), ferric chloride (200µl; 100mM), H_2O_2 (100µl; 200mM) and ascorbic acid (100µl; 300mM). This mixture was kept for 1h at 37°C. Then, trichloroacetic acid (1ml; 2.8%w/v) and thiobarbituric acid (1ml; 1%w/v) prepared in NaOH (50mM) were added to it and the whole reaction mixture was kept (15min) in water bath for boiling. After cooling, the absorbance of reaction mixture was measured spectrophotometrically (532nm). Normal and standard control (Quercetin) were prepared. Inhibition of deoxyribose degradation was calculated by

$$\% \text{ inhibition} = \frac{\text{Control Abs.} - \text{Sample Abs.}}{\text{Control Abs.}} \text{ X } 100$$

***In vitro* anti-inflammatory activity**

Anti-inflammatory activity of all the extracts was evaluated by different approaches viz., inhibition of protein denaturation, proteinase inhibitory activity and human red blood cell (HRBC) membrane stabilization assay in reference with the previous literatures.[14-17] All assays were done in triplicate and the results were expressed as mean ± standard deviation.

Inhibition of protein denaturation

Reaction mixture (0.5ml) was prepared using bovine serum albumin (0.45ml; 5% aq. solution) and plant extracts (0.05ml) in different concentration (50, 100, 150, 200µg/ml). pH of the reaction mixture was adjusted to 6.3 using 1N HCl. Distilled water was used as normal control and ibuprofen was employed as standard control. The reaction mixture was incubated at 37°C for 20min and then heated to 57°C for 20min. After cooling, 2.5ml phosphate buffer saline (pH 6.3) was added to each tube and the absorbance was measured spectrophotometrically (660 nm). The percentage inhibition of protein denaturation was calculated by

$$\% \text{ inhibition} = \frac{\text{Control Abs.} - \text{Sample Abs.}}{\text{Control Abs.}} \text{ X } 100$$

The concentration (µg/ml) of the drug required to denature 50% protein was calculated from the graph.

Proteinase inhibitory activity

The reaction mixture (2ml) composed of 0.06mg trypsin, 1ml of 20 mM Trypsin HCl buffer (pH 7.4) and 1 ml test sample of different concentration (50, 100, 150 and 200µg/ml) was incubated at 37°C for 5min and then 1ml of 0.8%w/v casein was added. The mixture was again incubated for additional 20min. 2ml of 70% perchloric acid was added to arrest the reaction. Cloudy suspension was centrifuged and the absorbance of the supernatant was read at 210nm against buffer blank. Ibuprofen was used as standard control. The percentage inhibition of proteinase inhibitory activity was calculated by

$$\% \text{ inhibition} = \frac{\text{Control Abs.} - \text{Sample Abs.}}{\text{Control Abs.}} \text{ X } 100$$

The concentration (µg/ml) of the drug required to denature 50% proteinase (IC_{50} value) was calculated from the graph.

HRBC membrane stabilization method

An equal volume of freshly collected whole human blood from the healthy volunteers and the sterile Alsever solution (2% dextrose, 0.8% sodium citrate, 0.05% citric acid, 0.42% sodium chloride/100ml distilled water) was mixed and subjected to centrifugation (3000rpm for 10min). The packed cells thus obtained was washed and reconstituted as 10%v/v suspension by using sterile isosaline (0.85% NaCl in H_2O sterilized by autoclaving). 1ml of HRBC suspension and 1ml of each test extracts in different concentration (50, 100, 150 and 200µg/ml) was taken in the individual tubes. Normal (HRBC suspension and Alsever solution only) and standard control (Ibuprofen instead of test extracts) were prepared. All the tubes were incubated for 30min at 37°C, followed by subjected to centrifugation. The supernatant was collected and its haemoglobin content was estimated spectrophotometrically (560nm) and the percentage haemolysis and protection were calculated by

$$\text{Percentage of haemolysis} = \frac{\text{OD of test}}{\text{OD of control}} \text{ x } 100$$

$$\text{Percentage of protection} = 100 - \frac{\text{OD of test}}{\text{OD of control}} \text{ x } 100$$

OD of test – Optical density of tested extracts; OD of control – Optical density of normal control.

RESULTS AND DISCUSSION

Processing and extraction of plant material

In the present study, the aerial parts of *E. scaber* was collected, dried in shade, made into coarse powder, extracted by soxhlation with solvents of increasing polarity viz., Petroleum ether, Chloroform, Ethyl acetate and Methanol. In the analysis of colour, consistency and percentage yield of extracts, it was found that all the extracts were waxy semisolid in consistency, in case of colour, all the extracts were brownish green in colour except the petroleum ether extract which was yellowish green in colour. The percentage yield analysis revealed that the petroleum ether extract gave 4.36%w/w, the chloroform extract gave 6.25%w/w, ethyl acetate and methanol extract gave 9.52 and 13.50%w/w of dried extract respectively (Table 1).

Table 1: Percentage yield in the extraction of aerial part of the *E. scaber*.

Extract	Extraction method	Colour of the dried extract	Physical nature of dried extract	% yield of the extract (%w/w)
Petroleum ether	Soxhlation (Successive solvent extraction)	Yellowish green	Waxy semisolid	4.36
Chloroform		Brownish green	Waxy semisolid	6.25
Ethyl acetate		Brownish green	Waxy semisolid	9.52
Methanol		Brownish green	Waxy semisolid	13.50

Preliminary phytochemical evaluation of prepared extracts

Table 2: Preliminary phytochemical evaluation of different extracts of *E. scaber*.

S. No.	Chemical Test	I	II	III	IV
1	**Alkaloids**				
a	Mayer's test	-	-	-	-
b	Wagner's test	-	-	-	-
c	Hager's test	-	-	-	-
d	Dragendorff's test	-	-	-	-
2	**Glycosides**				
a	Legal's test	+	+	+	+
b	Baljet's test	+	+	+	+
d	Borntrager's test	-	-	-	-
e	Modified Borntrager's test	-	-	-	-
3	**Phenolic compounds**				
a	Ferric chloride test	+	+	++	++
b	Lead acetate test	+	+	++	++
c	Gelatin test	+	+	++	++
4	**Flavones and flavanoids**				
a	Aqueous NaoH test	+	+	++	++
b	Ammonia test	+	+	++	++
c	Shinoda test	+	+	++	++
5	**Carbohydrates**				
a	Molisch's test	+	+	+	+
b	Fehling's test	+	+	+	+
c	Benedict's test	+	+	+	+
6	**Proteins and Amino acids**				
a	Millon's test	-	-	+	+
b	Biuret test	-	-	+	+
c	Ninhydrin test	-	-	+	+
7	**Terpenoids**				
a	Salkowski's test	+	++	++	++
8	**Sterols**				
a	Libermann-Burchard's test	+	+	++	++
b	Salkowski's test	+	+	++	++
9	**Saponins**				
a	Foams test/froth test	+	+	+	++
b	Haemolysis test	+	+	+	++
10	**Gum & mucilage**	-	-	-	-
11	**Volatile oil**	-	-	-	-

I – Petroleum ether extract; **II** – Chloroform extract; **III** – Ethyl acetate extract; **IV** – Methanol extract; (+) – Positive result; (++) – Significant positive result; (-) – Negative result.

Table 2 shows the results of qualitative phytochemical analysis of the prepared extracts for the identification of various phytoconstituents. The tests for alkaloids, gum and mucilage and volatile oil gave negative results in all the employed tests. The presence of glycosides and carbohydrates were found in all the tested extracts. Phenolic compounds, flavonoids and sterol were found in all the tested extracts, particularly, the ethyl acetate and methanol extract showed a significant presence. Saponin was found in all the tested extracts, particularly, the methanol extract showed a significant presence. In case of terpenoids, all the tested extracts showed a significant presence except the petroleum ether extract. The ethyl acetate and methanol extract showed the presence of proteins and amino acids.

Results of preliminary phytochemical evaluation of the present study is in accordance with the previous

findings.[18-22] These literatures revealed the presence of different category of phytoconstituents in the extracts of *E. scaber* (Figure 1). Notably, findings of the present study also agrees with these previous reports.

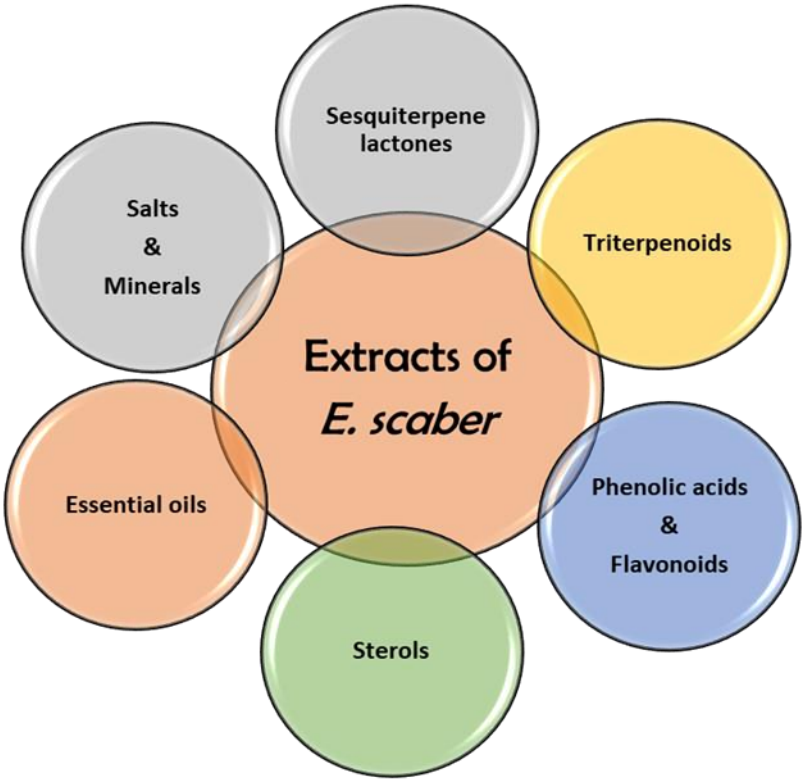

Figure 1: Major phytoconstituents of *E. scaber*.

Kabeer FA & Prathapan R 2014[20] stated that phenolic acids occur in nature in both free and bound forms, including esters and glycosides. These phenolic compounds vary from simple low-molecular weight substances, such as phenylpropanoids, coumarins, and derivatives of benzoic acid, to more intricate structures like flavonoids, stilbenes, and tannins. Various fractions of the entire plant *E. scaber* have yielded phenolic acids and flavonoids. Results of the present study also indicated a significant presence of phenolic compounds and flavonoids.

The sesquiterpenoids are C_{15} compounds formed by the assembly of three isoprenoid units. Their structure may be linear, monocyclic or bicyclic. They constitute a very large group of secondary metabolites. Based on their carbocyclic skeletons, sesquiterpene lactones can be classified in to four major groups – Germacranolides, Guaianolides, Pseudoguaianolides and Eudesmanolides. All sesquiterpene lactones contain alpha-methylene gamma-lactone ring either cis-or-trans fused to the C_6-C_7 or C_8-C_7 position of the carboxylic skeleton. Presence of sesquiterpene lactones, triterpenes and steroids in the extracts of *E. scaber* were reported in the published documents, also, essential oil, salt and minerals were reported[20-22] Results of the present study also indicated a significant presence of sesquiterpene lactones, triterpenes and steroids. Based on the findings in the preliminary phytochemical evaluation, it was decided to evaluate the *in vitro* anti-oxidant and anti-inflammatory activity of the extracts of *E. scaber*.

***In-vitro* antioxidant activity**

In-vitro antioxidant activity of the all the prepared extracts viz., petroleum ether, chloroform, ethyl acetate and methanol extracts of *E. scaber* were evaluated by DPPH, reducing power assay, superoxide, nitric oxide and hydroxyl free radical scavenging assay methods.

The results of evaluation by DPPH method is shown in Table 3. In this assay, all the tested extracts showed a concentration-dependent rise in activity. Generally, all the tested extracts revealed the highest percentage of inhibition at the concentration of 200µg/ml, the highest one employed for the evaluation. Among all the test extracts, the methanol extract showed the highest percentage (89.54%) of inhibition at the concentration of 200µg/ml which is comparable with the score of ascorbic acid (95.11%). Next to that, the ethyl acetate extract showed a percentage inhibition of 83.40%.

The next method employed for the evaluation of antioxidant activity, the reducing power assay method also showed a concentration dependent increase of antioxidant activity in all the tested extracts. In this method also, the methanol extract proved its antioxidant potential by showing a percentage inhibition of 85.10±1.65 followed by the ethyl acetate extract showed

a percentage inhibition of 76.70±1.20. Both these two extracts of *E. scaber* revealed a significant antioxidant activity comparing with the standard agent, ascorbic acid, employed for the comparative evaluation (Table 4).

Table 3: Antioxidant activity of test extracts by DPPH assay.

Con.	% Inhibition				
	Test extracts				
(µg/ml)	Pet. ether	Chloroform	Ethyl acetate	Methanol	Std.
50	41.05±0.10	44.15±0.30	46.20±1.36	55.31±1.68	67.45±0.13
100	49.20±0.25	54.37±0.10	58.19±1.27	63.05±1.55	79.51±1.63
150	61.14±0.45	63.12±0.57	71.50±1.17	77.24±1.80	86.24±1.42
200	74.11±0.57	77.10±1.09	83.40±1.60	89.54±2.07	95.11±2.50
IC_{50}	112.43	110.50	104.20	91.25	75.29

Con. – Concentration; Std. – Standard (ascorbic acid)

Table 4: Antioxidant activity of test extracts by reducing power assay.

Con. (µg/ml)	% Inhibition				
	Test extracts				
	Pet. ether	Chloroform	Ethyl acetate	Methanol	Std.
50	15.10±0.14	26.70±0.60	38.65±1.20	45.28±1.15	49.54±0.48
100	26.30±0.37	33.95±0.82	47.76±2.15	58.50±1.41	61.25±0.65
150	35.60±1.22	51.36±1.16	64.50±1.55	66.40±1.39	73.15±0.58
200	47.09±1.45	65.10±1.22	76.70±1.20	85.10±1.65	94.23±1.13
IC_{50}	135.60	121.50	114.25	101.30	85.50

Con. – Concentration; Std. – Standard (ascorbic acid)

A similar kind of results was found in superoxide radical scavenging assay, another one method employed for assessing the antioxidant ability of the extracts of *E. scaber*. In this method also, comparing with other tested extracts and the standard agent (ascorbic acid), the methanol extract revealed a maximum activity by showing a percentage inhibition of 64.30±0.62 in 200µg/ml, the highest concentration of evaluation (Table 5).

Table 5: Antioxidant activity of test extracts by superoxide radical scavenging assay.

Con. (µg/ml)	% Inhibition				
	Test extracts				
	Pet. ether	Chloroform	Ethyl acetate	Methanol	Std.
50	12.31±0.68	15.23±1.05	21.44±0.14	29.31±0.50	49.37±0.25
100	17.28±0.57	24.12±0.10	28.18±0.30	36.40±0.20	61.50±0.45
150	23.10±0.79	29.55±0.44	37.28±0.76	49.25±0.35	75.17±0.13
200	31.46±0.29	37.10±1.20	45.15±0.20	64.30±0.62	83.07±0.17
IC_{50}	125.10	113.25	105.33	95.50	84.20

Con. – Concentration; Std. – Standard (ascorbic acid)

The other two methods, nitric oxide and hydroxyl radical scavenging assay utilized for the evaluation of antioxidant activity also revealed a same sort of results (Table 6 & 7). Results of these evaluations clearly indicated the antioxidant potential of methanol extract comparing with other tested extracts and standard agent (ascorbic acid in nitric oxide radical scavenging assay and quercetin in hydroxyl radical scavenging assay) used for the comparative evaluation.

Table 6: Antioxidant activity of test extracts by nitric oxide radical scavenging assay.

Con. (µg/ml)	% Inhibition				
	Test extracts				
	Pet. ether	Chloroform	Ethyl acetate	Methanol	Std.
50	24.30±0.50	29.16±1.20	37.22±0.43	45.58±0.60	59.39±0.80
100	36.40±0.12	38.40±0.81	49.63±0.25	57.35±0.40	67.50±0.30
150	43.20±0.30	51.60±0.73	63.19±0.56	69.19±0.67	79.22±0.12
200	55.26±0.55	65.23±0.52	75.20±0.16	85.29±0.53	91.58±0.40
IC_{50}	121.76	115.69	106.85	95.01	67.36

Con. – Concentration; Std. – Standard (ascorbic acid)

Table 7: Antioxidant activity of test extracts by hydroxyl radical scavenging assay.

Con. (µg/ml)	% Inhibition				
	Test extracts				
	Pet. ether	Chloroform	Ethyl acetate	Methanol	Std.
50	14.20±0.35	23.56±1.10	35.22±0.45	47.18±0.50	58.29±0.70
100	23.15±1.16	37.20±0.71	46.33±0.15	59.25±0.30	65.40±0.20
150	37.20±0.74	49.30±0.53	57.16±0.36	73.29±0.57	79.12±0.22
200	46.20±0.55	61.73±0.22	71.20±0.30	87.49±0.63	95.50±0.30
IC_{50}	134.16	125.39	110.15	91.50	87.16

***In vitro* anti-inflammatory activity**

It was done by inhibition of protein denaturation, proteinase inhibitory activity, HRBC membrane stabilization. In the inhibition of protein denaturation assay, the results showed a concentration-dependent rise of activity which was clearly indicated by the methanol extract (200µg/ml) with the percentage inhibition of 79.50±1.03, followed by the ethyl acetate extract in the same concentration revealed percentage inhibition of 71.48±1.50 (Table 8).

Table 8: *In vitro* anti-inflammatory evaluation of tests by protein denaturation inhibition method.

Conc. (µg/ml)	% inhibition				
	Std. (Ibu.)	Test extracts			
		1	2	3	4
50	49.24±0.47	25.60±0.71	29.50±0.85	35.29±1.30	43.05±1.51
100	61.85±0.36	36.14±0.54	41.31±0.46	46.34±1.09	59.28±0.95
150	75.39±0.79	44.15±0.50	53.25±0.30	55.28±1.20	67.42±0.30
200	86.50±1.35	57.81±1.01	66.74±1.65	71.48±1.50	79.50±1.03
IC_{50}	56.37	231.47	125.60	116.50	87.15

Conc. – Concentration; Std. – Standard (Ibuprofen); 1- Petroleum ether; 2- Chloroform; 3-Ethyl acetate; 4- Methanol

Proteinase inhibitory activity, another one method employed for the evaluation also showed a concentration-dependent rise of activity. In this method also, the methanol extract in 200µg/ml concentration showed the maximum percentage inhibition of 71.98±0.08, followed by the ethyl acetate extract in the same concentration showed 62.13±0.10 as percentage inhibition (Table 9).

Table 9: *In vitro* anti-inflammatory evaluation of tests by proteinase inhibitory activity.

Conc. (µg/ml)	% inhibition				
	Std. (Ibu.)	Test extracts			
		1	2	3	4
50	45.33±0.28	13.17±0.92	23.14±0.50	35.79±1.42	39.54±0.35
100	57.84±0.35	21.56±0.80	35.62±0.37	41.64±1.63	45.17±0.62
150	69.05±0.40	29.06±0.65	44.13±0.62	49.32±0.09	57.04±0.19
200	81.10±0.05	47.36±0.09	56.29±0.10	62.13±0.10	71.98±0.08
IC_{50}	68.51	216.83	159.67	126.21	95.00

Conc. – Concentration; Std. – Standard (Ibuprofen); 1- Petroleum ether; 2- Chloroform; 3-Ethyl acetate; 4- Methanol

In HRBC membrane stabilization method also a concentration-dependent rise of activity was found. In this method also, the methanol extract showed a significant score of percentage protection comparing with other tested extracts. It showed 63.23±1.58 as percentage protection score, next to that the ethyl acetate extract showed 55.49±1.95 as percentage protection (Table 10). These findings are comparable with the score showed by the standard drug ibuprofen which showed 81.20±1.65 as percentage protection.

Table 10: Effect of test extracts on the protection of HRBC membrane.

Conc. (µg/ml)	% Protection				
	Std. (Ibu.)	Test extracts			
		1	2	3	4
50	52.22±1.30	15.31±0.28	17.45±0.92	21.54±0.72	28.36±1.56
100	55.30±1.50	21.89±0.58	23.36±1.01	35.20±0.14	39.51±2.07
150	66.11±0.26	31.67±0.67	36.52±0.53	46.58±1.55	49.25±2.56
200	81.20±1.65	38.69±1.12	45.21±2.32	55.49±1.95	63.23±1.58
IC_{50}	75.21	210.13	149.37	120.10	105.00

Conc. – Concentration; Std. – Standard (Ibuprofen); 1- Petroleum ether; 2- Chloroform; 3-Ethyl acetate; 4- Methanol

CONCLUSION

The phytochemicals found in the preliminary phytochemical evaluation of the present study may responsible for the biological activities revealed by the extracts. In summary, this study demonstrated that the solvent methanol yielded noteworthy results, evident from the outset, including the extraction yields and the various pharmacological activities assessed. Following the methanol extract, the ethyl acetate extract also exhibited significant findings across all aforementioned parameters. Future research concentrating on in vivo investigations may yield even more substantial results.

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