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In- Vitro Study of Anti-Oxidant and Anti-Inflammatory Activity on Roselle Flower

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ABSTRACT

Roselle (Hibiscus Sabdariffa) is the most important genera of the family Malvaceae, which is widely distributed all over the world and is used for centuries in the local medicine system. The main aim of this study was to investigate the phytochemical screening, in vitro antioxidant, and in vitro anti-inflammatory activities of crude methanolic extract of Roselle Flower. The results of phytochemical screening have shown that the plant extract has a detectable number of bioactive constituents: flavonoids, tannins, phenols, quinones, saponin, and terpenoids. The results of antioxidant activity of plant extract have shown that the alcoholic extract of leaves of this plant possesses better antioxidant potential when compared to reference standard ascorbic acid. Also, the result of in vitro anti-inflammatory activity via Egg Albumin protein denaturation method has been compared with standard show desired percentage inhibition The results obtained in the present study suggest that Flowers of Hibiscus Sabdariffa can be used as a source of functional ingredients for pharmaceutical drug industries.

Keywords: Antioxidant, Anti-inflammatory, Phytochemical tests, Plant extract.

1. INTRODUCTION

Hibiscus sabdariffa, commonly known as Roselle or Rosella, is a tropical plant widely cultivated for its edible calyces, which are rich in bioactive compound. Traditionally used in folk medicine across Asia, Africa, and the Caribbean. Rosella has generated increasing scientific interest for its potential health benefits, particularly its antioxidant and anti-inflammatory properties. The flower of H. Sabdariffa contains high level of phenolic compounds, flavonoids, and organic acids such as hibiscus acid. Roselle is a short day, annual herbaceous, bast fibre-bearing plant belonging to the Malvaceae family. Today roselle flower us attracting of food and beverage manufacturers and pharmaceutical concerns who feel it may have exploitable possibilities as a natural food product for herbal medicine and as a colorant to replace some synthetic dyes.[1]

2. ANTIOXIDANT ACTIVITY

Roselle is a medicinal plant widely recognized for its vibrant red calyces and rich phytochemical profile. Traditionally used in herbal medicine, Roselle has gained attention in modern pharmacological research for its strong antioxidant potential.[2] Flower contains high concentrations of phenolic compounds, flavonoids, anthocyanins- such as delphinidin and cyanidin derivatives- that act as powerful free radical scavengers. These antioxidants helps in neutralize reactive oxygen species. In-Vitro assays such as DPPH[2,2-diphenyl-1-picrylhydrazyl] is commonly used to evaluate the antioxidant capacity of Roselle flower extracts. These tests measure the ability of Roselle to donate electron or hydrogen atoms, indicating its free radical neutralizing potential.[3]

3. ANTI-INFLAMMATORY ACTIVITY

Roselle is a medicinal plant traditionally used to treat various inflammatory condition.[4] Recent pharmacological studied have highlighted its significant anti-inflammatory properties. Roselle flower are rich in bioactive compounds such as flavonoids, anthocyanins and phenolic acids.[5] These constituents are known to modulate inflammatory responses by inhibiting pro-inflammatory mediators. Rosella extract has been shown to suppress the production of nitric oxide (No) and pro-inflammatory cytokines like TNF alpha and IL-6 in lipopolysaccharide(LPS)- stimulated macrophage cell lines, such as RAW 264.7 cells. It suggest that Roselle can inhibit key pathway like NF-KB and COX-2, which are crucial in inflammation signaling.[8]

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4. PLANT PROFILE:

Figure no 1: Hibiscus Sabdariffa Linn (Roselle Plant)

Scientific Name	Hibiscus Sabdariffa Linn
Kingdom	Plantae
Family	Malvaceae
Genus	Hibiscus
Species	Hibiscus Sabdariffa
Phylum	Spermatophyta (Flowering Plant)
Sub-division	Angiospermae
Class	Dicotyledons
Order	Malvale

5. METHODOLOGY

Fresh Flowers of the Roselle plant was collected from a local region, Dried in shade for up to 3-4 days. The plant material was identified by via verification and Authentication by Dr. Biradar sir, Plant Taxonomists, Shivaji College, Udgir. Dist. Latur.

5.1 Extraction

Simple Maceration Method:

Roselle was cleaned and rinsed thoroughly with distilled water, and then flowers were dried in shade for 3-4 days. The dried flowers were crushed to make fine powder and then the powder was used for maceration. The maceration method was followed for the extraction.

15-20 grams powder of dried flowers was added to 100 ml of 80 % methanol in a flask (250 ml capacity) the maceration process was carried out for 3 days and kept in a dark place at room temperature. After incubation, the macerated sample was filtered by using Satmed Whatman filter paper grade 01. The supernatant was kept for evaporation in a glass Petri dish for 3 days at room temperature. After evaporation of the solvent, the dried methanolic extract was scratched by a spatula. The dried methanolic extract was used for phytochemical screening, antioxidant and anti-inflammatory activity.

5.2 Phytochemical Screening

Test	Observation	Conclusion
B. Test for Flavonoids: 1 mL of plant extract (50 mg/mL) was added into 1 mL of 10% lead acetate solution.	Yellow coloured precipitate is formed.	Indicates presence of flavonoids.
C. Test for Phenol: 1 mL of plant extract (50 mg/mL) was added into 0.5 mL of 10% ethanolic ferric chloride solution.	Blue green to dark bluecoloration formed.	Indicates presence of phenols.
D. Test for Tannin: A few drops of 1% lead acetate were added to 2 mL of plant extract (50 mg/mL).	Dark blue or greenish grey coloration is observed.	Indicates presence of tannins.
E.Test for Terpenoids: 2.5 mL of plant extract (50 mg/mL) was mixed with 1 mL of chloroform and then 1.5 mL of concentrated H_2SO_4 was added.	Reddish-brown colour formed at the interface.	Indicates presence of terpenoids.
F. Test for Saponins: 5 mL of purified distilled water was taken into a test tube, followed by the addition of 500 µL of plant extract (50 mg/mL), and then the mixture was well stirred.	Continuous effervescence observed.	Indicates presence of saponins.
G. Test for Quinones: 1 mL of concentrated sulphuric acid was taken into a test tube, followed by the addition of 1 mL of plant extract (50 mg/mL).	Red colorationis observed.	Indicates presence of quinones.

6. MATERIAL AND METHOD

6.1 List of Chemical

Table no 1: List of Chemical

Sr. No.	Chemical Name	Uses
1	Ascorbic acid	Act as antioxidant
2	Sodium nitroprusside	Regulates antioxidant capacity
3	Sulphinilic acid	Coupling agent
4	2-naphthylamine	As antioxidant
5	Egg albumin	Protein source
6	Diclofenac sodium	Reduce inflammation
7	Phosphate buffer (6.3)	Maintain PH

6.2 Antioxidant Activity:

Total anti-oxidant capacity

Add 0.1 ml extract to 1 ml reagent solution (0.6 M Sulphuric acid, 28 mM Sodium phosphate, 4mM Ammonium Molybdate). Check the absorbance at 695 nm on UV visible spectrometer. [10]

6.3 Anti-Inflammatory Activity (Protein Denaturation) Method:

EGG'S-ALBUMIN

Test solution:

The reaction mixture (5 ml) consisted of 0.2 ml of egg albumin (from fresh hen's egg), 2.8 ml of phosphate-buffered saline (PBS, pH 6.3), and 2 ml of varying concentrations (2, 4, 6,8,10 µg/ml) of the test sample. Control solution: 5ml consists of 0.2 ml of egg albumin (from fresh hen's egg), 2.8 ml of phosphate-buffered saline (PBS, pH 6.3), and distilled water volume of up to 5ml.

Standard solution: (5ml) consists of 0.2 ml of egg albumin (from fresh hen's egg), 2.8 ml of phosphate-buffered saline (PBS, pH 6.3), and 2 ml of varying concentrations (2,4,6,8,10 µg/ml) of Diclofenac sodium drug.

Procedure: All sample tubes were incubated at 37±2°C in a BOD incubator for 15minutes and then extend to 70°C for five minutes. After cooling, their absorbance was measured at 660 nm by using the vehicle. [11]

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7. RESULT AND DISCUSSION

7.1 Phytochemical Screening

Table No 2. Phytochemical Screening

Organic compounds	Roselle *(Hibiscus Sabdariffa)*
Alkaloids	Absent
Flavonoids	Present
Phenol	Present
Tannins	Present
Terpenoids	Present
Volatile oil	Present
Saponins	Present
Quinones	Present

7.2 Total Antioxidant Method

Table no 3. Total Antioxidant Method

Concentration	Absorbance (nm)		% Inhibition	
	Test	Standard (Ascorbic acid)	Test	Standard (Ascorbic acid)
50 µg/ml	0.128	0.192	66.58	49.75
100µg/ml	0.270	0.256	28.91	32.11
150µg/ml	0.272	0.288	28.68	23.96
200µg/ml	0.298	0.331	21.32	13.17
250µg/ml	0.333	0.357	12.90	6.32
Control (Methanol)	0.378			

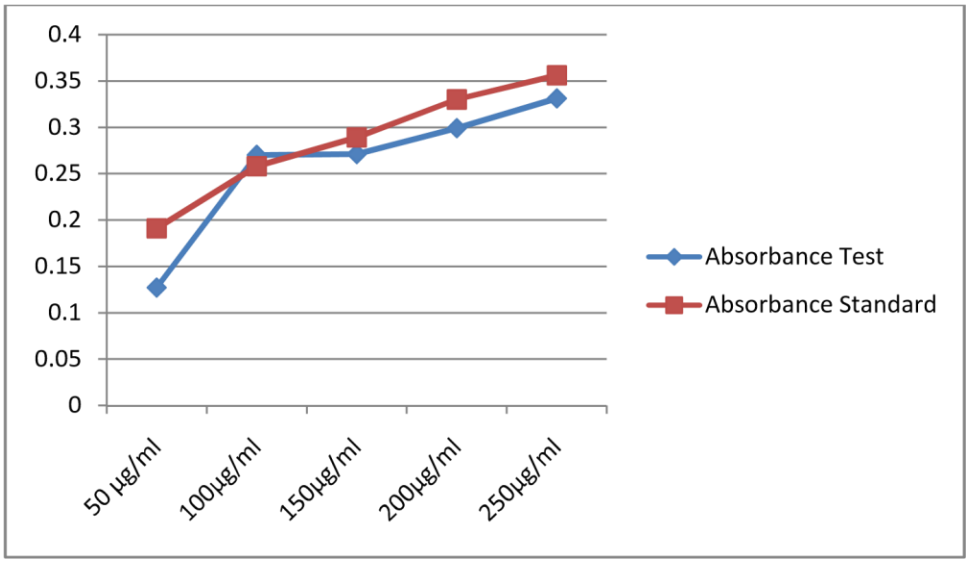

Figure no 2. Total Antioxidant Capacity Graph.

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7.3 Denaturation of Egg Albumin

Table no 4. Denaturation of Egg Albumin

Concentration	Absorbance (nm)		% Inhibition	
	Test	Standard (Diclofenac sodium)	Test	Standard (Diclofenac sodium)
2 µg/ml	0.037	0.061	73.6	56.96
4 µg/ml	0.064	0.096	54.87	34.04
6 µg/ml	0.099	0.072	31.93	50.68
8 µg/ml	0.077	0.046	45.6	67.37
10 µg/ml	0.064	0.077	54.7	47.25
Control (Egg Albumin)	0.156			

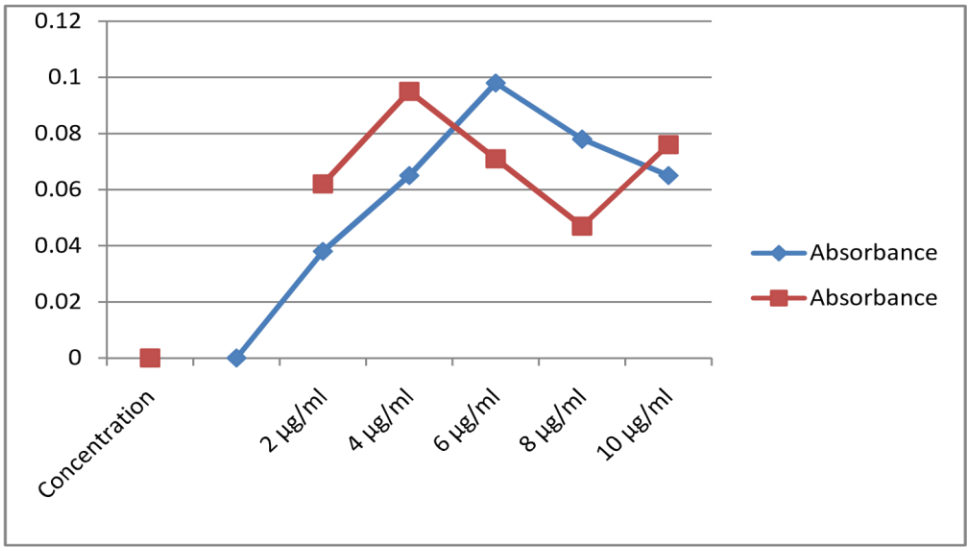

Figure no 3. Denaturation of Egg Albumin Graph.

8. CONCLUSION

This study's primary goal was to examine the crude methanolic extract of Roselle (Hibiscus Sabdariffa) flower's phytochemical screening, in vitro antioxidant, and in vitro anti-inflammatory properties.

Based on this experiment, it can be said that Hibiscus Sabdariffa flower crude methanolic extract exhibits both anti-inflammatory and anti-inflammatory properties. Hibiscus Sabdariffa flowers are abundant in phytochemical components, such as phenol, which has anti-oxidant properties. A secondary antioxidant is tannin. Quinine, cardiac glycoside, and saponin all exhibit anti-inflammatory properties. Terpenoids and resins exhibit anti-inflammatory and antioxidant properties.

Therefore, based on this experiment, it can be said that the crude methanolic extract of Hibiscus Sabdariffa flower has anti-inflammatory action (denaturation of protein using egg albumin) as well as antioxidant activity (Nitric oxide, total antioxidant capacity method). Roselle flowers are abundant in phytochemical components, such as phenol, which has anti-oxidant properties. One type of secondary antioxidant is tannin. Quinine and saponin exhibit anti-inflammatory properties. Terpenoids exhibit anti-inflammatory and antioxidant properties.

Roselle flowers were utilized as an anti-inflammatory and antioxidant.as an anti-diarrheal, sedative, liver illness, stress ulcers, and muscle stress reliever. In all of this method Total Antioxidant Capacity (Antioxidant Activity) is best method which shows near about results for test 0.271 and for standard ascorbic acid is 0.289 of 150µg/ml at 660nm. Flowers of Hibiscus Sabdariffa was also used as antioxidant and anti-inflammatory agent like in improving muscular stress, in liver diseases, stress ulcers, as anti-diarrhoeal and as a sedative.

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Conflict of Interest Statement: All authors have nothing else to disclose.

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