



PHYTOCHEMICAL COMPOUNDS ANALYSIS AND ANTIMICROBIAL ACTIVITY IN HIBISCUS SABDARIFFA L. (ROSELLE) LEAVES.

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ABSTRACT:

Hibiscus sabdariffa L. commonly named as “Roselle” is a medicinal plant belongs to the family Malvaceae. Phytochemical and biochemical compounds accumulated are used for different biological function. These phytochemicals have a salutary effect when consumed by the humans and used to treat different human diseases. The phytochemical compounds are extracted from plant by soxhlet hot percolation method by using methanol as a solvent. This plant has anti cancerous, analgesic, anti-inflammatory, antihypertensive, antitumor, and antiviral. The result of Roselle leaves extract contain phytochemical with antimicrobial and anti-oxidative properties.

INTRODUCTION:

Olden day's humans used to use medicinal plants to cure some diseases. Ethno botanical studies have revealed the importance of plants in treating infectious diseases (1). Plants contain antimicrobial compounds such as polyphenols, phenolic, terpenoids, essential oils, lectins and alkaloids (2). These compounds act on the bacteria through different mechanism: phenolic compounds modify the permeability of cell membrane, tannins inactive the metabolism by binding the enzymes, phenolic acid disrupts the membrane integrity and leakage of essential intracellular constituents, Flavonoids also may act through inhibiting both energy metabolism and DNA synthesis thus affecting protein and RNA syntheses (3). Secondary metabolites isolated from the medicinal plants have anti cancerous, antibacterial, analgesic, anti-inflammatory, antihypertensive, antitumor, and antiviral and many other activities (4).

Antioxidants are those substances that prevent most of the oxidation reaction which are initiated with the production of free radicals, it prevent the damage to the tissue and cells of living organisms and also known as reducing agents. It is used in cosmetics, food preservation, prevention of gasoline, rubber degradation. Phytochemical compounds like flavonoids, lignin's, tannins, and phenolic compounds represent antioxidant potentials (5).

Hibiscus sabdariffa L. belongs to the family Malvaceae also known as Roselle or Sorrel or Zobo plant is a native to Asia (India to Malaysia) or tropical Africa. The plant is widely distributed in India, Caribbean Africa, and Central America, Brazil etc. In Indian languages it is known as Lalambaris patwa (Hindi), Pulachakiri, Pundibija (Kannada), Gongura, Yerragogu (Telugu), Polechi, Pulichchai (Malayalam), Lalmistachukas (Bengali), Lalambadi (Marathi) and Chukkaiair (Assam) (6).

“Roselle” grows in a bush with many branches. The flowers of Roselle are axillaries or in terminal racemes, the petals are white with reddish center at the base of the stamina column and this species is widely used as food. The young leaves and tender stem is consumed as a green vegetable used in salads, soups. Calyces used in herbal tea, present in soft drinks and use as a food preservative. Seeds are very rich in proteins, fat and total sugars (7). This plant is used herbal medicine to treat sore throat and for wound healing.

Investigations revealed that plant is highly rich in vital mineral and nutrients such as Iron, Copper, Calcium, Magnesium, Manganese required for the healthy growth in humans (8).

MATERIALS AND METHODS:

Preparation of extracts:

The leaves are washed under tap water and rinsed with distilled water three times and dried in the shaded area and powered by using pestle and mortar, the powder is packed in the soxhlet apparatus to continuous hot percolation using methanol as a solvent. The extract was concentrated and dried, powered sample stored in refrigerator at 4°C (9).

Preliminary Phytochemical Screening:

The methanol extract of the leaves was used for the preliminary phytochemical screening procedure for the presence of bioactive ingredients such as tannins, alkaloids, flavonoids, saponins, and steroids (10).

Phytochemical analysis:

The methanolic extraction of leaves sample was tested for phytochemical compounds.

a. Test for alkaloids:

The extract was evaporated to dryness and the residue was heated on a boiling water-bath with 2% Hydrochloric acid. After cooling, the mixture was filtered.

- Meyers Reagent - It is treated with a few drops of Meyer's reagent. The samples were then observed for the presence of turbidity or yellow precipitation (11).
- Wagner's test - A few drops of Wagner's reagent are added to a few amount of plant extract and a reddish brown precipitate depicts the presence of alkaloids (12).
- Dragendroff's test - The addition of few drops of Dragendroff's reagent into the extract gives red precipitate if alkaloids are present in the sample (12).
- Hager's test - A small amount of Hager's reagent is added to the extract. The formation of yellow precipitate indicates the presence of alkaloids (12).

b. Test for flavonoids:

- Alkaline reagent test - The plant extract was taken in the test tube and added few drop of dilute sodium hydroxide solution. If intense yellow colour appears in the test tube. It became colorless when on addition of a few drop of dilute acid this indicates the presence of flavonoids (13).
- Magnesium and HCl test – the plant extract was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was integrated. To this solution, 5-6 drops of concentrated hydrochloric acid was integrated and development of orange or red color indicates the presence of flavonoids (11)
- Acid reagent test - One to five drops of concentrated hydrochloric acid (HCL) were added to little amount of plant extract. Immediate development of red colour indicates presence of flavonoids (14).

c. Test for tannins:

- 5mg of extract was placed in a test tube and then 2ml of 5% of ferric chloride (FeCl₃) solution added. A greenish black colour indicates the presence of tannins (14)
- A few drops of 1% gelatin solution containing sodium chloride are added to the plant extract. The formation of white precipitate indicates the presence of tannins (12).

d. Test for saponins:

- 5 mg of extract was shaken vigorously with 5 ml of distilled water in a test tube and heated. The formation of stable foam was accepted as an indication of the presence of saponins (11).

e. Test for steroids:

- 1mg of plant extract is taken to the 10ml of chloroform is added and equal amount of concentrated sulphuric acid at the side wall of the test tube. If the upper layer of the test tube turns red, sulphuric acid layer turns yellow with green fluorescence; it indicates the presence of steroids (13).

f. Test for proteins and amino acids:

- Biuret test - One drop of 2% copper sulphate solution is added to 2 ml of filtrate. Then 1 ml of 95% ethanol is added following by excess of potassium hydroxide pellets. Pink color in ethanolic layer indicates the presence of proteins (12).
- Ninhydrin test - Two drops of ninhydrin solution are added to 2 ml of the filtrate and purple color proves the presence of amino acids (12).
- Xanthoproteic test – the extract treated with few drops of concentration nitric acid. Formation of yellow color indicates presence of proteins (15).

g. Test for triterpenoids :

- The dry crude plant extract (5 mg) was dissolved chloroform (2 mL) and then acetic anhydride (1 mL) was added to it. Concentrated sulphuric acid (1 mL) was added to the solution. Formation of reddish violet colour shows the presence of triterpenoids (13).
- Extract treated with chloroform and filtered. The filtrate treated with few drops of concentrated sulphuric acid, shaken well and allows it to stand if golden yellow color appears it indicates the presence of triterpenes (15).

h. Test for carbohydrates:

Extracts were dissolved in 5ml distilled water and filtered; filtrate is used for carbohydrate analysis (15).

- Molisch's test - A few drops of sample solution is taken in a clean and dry test tube. Then a few drop of α -naphthol solution is added to it and shaken carefully. Finally conc. H_2SO_4 is poured into the test tube slowly. A violet ring or purple violet coloration appears this indicates the presence of carbohydrates.
- Benedict's test – 3ml of extract add 2-3ml of benedicts reagent and boil for 5mins if appearance of green or yellow or red, this indicates the presence of carbohydrates.
- Silver precipitation method – 5ml of plant extract to that a drop of ammonium hydroxide and excess of silver nitrate is added, if any formation of white precipitate this indicates the presence of carbohydrates.

i. Detection of phenols :

- The plant extract is treated with few drops of ferric chloride solution and the formation of bluish black color proves the presence of phenols (12).

j. Detection of phytosterols:

- Extract treated with chloroform and filtered. The filtrate treated with few drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid is added formation of brown ring at the junction; it indicates the presence of phytosterols.

k. Detection of fixed oils and fats:

- Stain test: A small quantity of extracts is pressed between two filter papers. An oily stain indicates presence of fixed oils.

l. Detection of Resins:

- Acetone – water test: plant extract is treated with acetone and small amount of water and it is shaken, appearance of turbidity indicates presence of resins.

ANTIBACTERIAL POTENTIAL:

The antibacterial potential of the methnolic extract using well diffusion method.

- Agar well Diffusion method.
24 hour old cultures are used for well diffusion method. One well of 6mm size was made in the help of sterile cork borer under aseptic condition in laminar air flow chamber. The wells were loaded with different concentrations such as 100mg/ml, 50mg/ml; 25mg/ml of the leaf extracts. The plates were incubated at 37°C for 24 hours. The plates were observed after 24 hours for clearing zone around the well. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (10).
- Disc diffusion method:
Bacteria are inoculated on the petri plate containing agar and incubated it for 24hrs. Filter paper discs (5mm) soaked in the plant extracts (20mg/ml) for 2hours and the discs were carefully placed on agar inoculated with bacteria. These plates were incubated at 37°C for 24 hrs. The plates were observed after 24 hours for clearing zone around the well. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well.

RESULT:

Hibiscus sabdariffa or Roselle leaves shows positive result for Flavonoids, tannins, saponins, steroids and negative result for alkaline. The antibacterial activity of methanolic extract of *Hibiscus sabdariffa L.* against *Salmonella typhi*, *Staphylococcus aureus* and *Escherichia coli*. The zone of inhibition diameter is high in *E.coli* (18mm) then in *Staphylococcus aureus* (17.5mm) and less in *Salmonella typhii* (8mm) at the concentration of 100mg/ml (10).

CONCLUSION:

Hibiscus sabdariffa contain bioactive components, potential source of drugs in herbal medicine so, it can be used as therapeutic compounds and it have antimicrobial properties so can be used in pharma industry for controlling infectious diseases.

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