E-ISSN 2250-0944
ISSN 2250-1150
doi: 10.31838/ijprt/12.01.04

Research Article

Antimicrobial and Antioxidant activity of Hibiscus Sabdariffa. linn (Roselle)

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Received: 19.07.21, Revised: 11.08.21, Accepted: 05.11.21

ABSTRACT
More than 300 species of Hibiscus Sabdariffa Linn. are present all over the world. Roselle is an annual herbaceous plant obtained from Hibiscus Sabdarifa Linn. Belonging to the family Malvaceae and Genus Hibiscus. It is also known as Aambadi in Marathi. Roselle is used for various medicinal purposes. Hibiscus Sabdariffa uses for making jellies, sauces, syrups, jams as well as use as a flavoring and colouring agent in drinks. the calyx is used as a beverage that helps to lower body temperature. Roselle is also used as a diuretic, laxative, anthelmintic. Also, use to lower blood pressure.15%-30% plant is made up of plant acids like citric acid, tartaric acid. Roselle mainly contains alkaloids, quercetin, L-ascorbic acid, anthocyanin, Beta-carotene. In this article, we focused on the antioxidant and antimicrobial activities of the rosella flower and we found that the alcoholic extract of the plant shows good antioxidant and antimicrobial activity as compared to the aqueous extract at very low concentration. The antioxidant activity was performed by using the DPPH and ferric thiocyanate activity model while the antimicrobial activity is done by using E.coli and S. aureus which shows a good result when they are evaluated.

Keywords: Hibiscus Sabdariffa linn, Antimicrobial, Antioxidant, DPPH, E.Coli.

INTRODUCTION

In Ayurveda and the traditional Chinese medicine system, it is well documented that the use of herbal extracts and nutritional supplements for the treatment of various diseases. The Roselle plant (Hibiscus sabdariffa) Linn (Malvaceae) has been used as a diuretic, mild laxative, and treatment for cardiac and nerve diseases.[1] In this article we discussed the antimicrobial and antioxidant activity of the various extract for the roselle plant. The Hibiscus has more than 300 species distributed in tropical and subtropical regions around the world and is used as ornamental plants. Research has shown that some species of Hibiscus possess certain medicinal properties of which Hibiscus sabdariffa linn is one. Hibiscus sabdariffa linn is commonly named as "red sorrel" or "roselle".[2] Even though permeable soil is the best, Roselle can Hibiscus sabdariffa Linn. also known as Roselle is one of the most important species of Hibiscus. The genus consists of about 300 species, some of which are widely distributed as tropical herbs and shrubs. Some of these are H. sabdariffa, H. cannabinus, H. tiliaceus, H. surattensis, H. acetosella, H. physaloides H. lunarifolius, and H. scotellii.[3] Hibiscus sabdariffa Linn is a species of hibiscus, native to the old-world tropics, used for the production of best fiber and as an infusion (herbal tea). The plant is an annual or perennial herb or woody-based sub-shrub, growing up to 2-2.5 m (7-8 ft.) tall. The leaves are deeply 35 lobed, 8-15 cm long arranged alternatively on the stems. The flowers are 8-10 cm in diameter, white to pale yellow with a dark red spot at the base of each petal, and have a stout fleshy calyx at the base 1-2 cm wide, enlarging to 3-3.5 cm, fleshy and bright red as the fruit matures. It takes about 6months to mature. The plant is widely cultivated for its strong fibers and it is well known for its edibility and medicinal properties, though the calyx is the most frequently used portion of the plant, the leaves and seeds are often made into salads, curries, and potherbs. They are rich in vitamins, natural carbohydrates, protein, tannins, gums, and other antioxidants including minerals. The calyx contains per 100 g, contained 49 calories, 84.5% water, 1.99 protein, 0.1 g fat, 12.3 g total carbohydrate, 2.3 g fiber, 1.2 g ash, 1.72 mg calcium, 57 mg phosphorus, 2.9 mg iron, 300 g vitamin A equivalent and 14mg ascorbic acid. [4]

The Roselle plant, with an attractive flower believed to be native to Africa, is cultivated in Sudan, India, Malaysia, and Taiwan. Roselle is cultivated in various parts of India. Mainly in Maharashtra, Panjab, Uttar Pradesh, Assam,

Andhra Pradesh, Madhya Pradesh, Bihar, Orrisa &West Bengal during April-November.[5]

This plant is used for mild laxative effect, the ability to increase urination, relief during hot weather, and treatment of cracks in the feet, bilious, sores, and wounds. Traditionally in Sudan, India, Roselle has been used for the relief of sour throat and healing wounds. In African as well as in Indian folk medicine, Roselle leaves are used for their, antimicrobial, emollient, antipyretic, diuretic, anti-helminthic, sedative, properties, and as a soothing cough remedy, whereas in India, leaves are potent for swelling containing fluid.[2]

The Hibiscus sabdariffa Linn leaves contain polyphenolic compounds like anthocyanins, protocatechuic acid, flavonoids like quercetin, kaempferol, hibiscetine, and sabdaretine. The major pigment is hibiscus. Apart from leaves, the seeds are also a good source of lipid-soluble antioxidants, particularly gamma-tocopherol.

Plant profile Hibiscus sabdariffa Linn

Synonym: - Carcade, Rozelle, Red Sorrel, Jamaica Sorrel, Indian Sorrel, Lal Ambadi, Gongura, Chukar.

Marathi:-Lal-Ambadi;

Bengali:-Lal-Mista;

Sanskrit:-Ambasthiki;

Hindi:-Patwa

Physical Properties

Colour: **Leaves** – Reddish green; **Stem**- Red; **Flower**- White to Pale yellow colour with a dark red spot at the base.

Odor: - Characteristic

Taste: - Sour, Slightly sweet

Size: - **Leaves**: 8-15 cm long ; **Flowers**: 8-10 cm in diameter; **Calyx**: 1-2 cm to 3.3.5 cm

Shape: - **Flowers**: Hibiscus – shape, Fleshy cup-shaped flowers.

Fig.1: Hibiscus Sabdariffa Linn.

MATERIALS AND METHODS

Plant material collection

The calyxes of the Hibiscus sabdariffa Linn. plant were collected from the herbal garden of Hi-Tech College of Pharmacy, Chandrapur, Maharastra, India. This collected material is then dried and store in an airtight container. The dried red Roselle (Hibiscus sabdariffa L.) calyces were collected, wash and dried in sunlight. 2 kg of calyx were ground by the electric grinder, and then samples were sieved and kept in polyethylene bags at 5°C in the refrigerator.

Chemicals

1, 1-diphenyl-2-picryl hydrazide (DPPH), bovine serum albumin, butylated hydroxytoluene (BHT) were purchased from Sigma Ltd. Folin Ciocalteu reagent; Ferric thiocyanate, 98 %Ethanol ,40 Mm Phosphate buffer solution (pH -7.0),75% Ethanol ,30% Ammonium thiocyanate,20 Mm Ferrous chloride,3.5% HCl ,1% Potassium ferricyanide ,1% Ferric chloride, linoleic acid solution. Bacterial isolated culture, Agar medium, methanol, penicillin (antibiotic). were purchased from Sd Fine Chemicals. All the other chemicals used were of AR grade.

Microorganism

These are two microorganisms that were used to check the antimicrobial properties of two organisms are gram-positive staphylococcus aureus and gram-negative E. coli.

METHODOLOGY

Preparation of extracts

Aqueous and Alcoholic were used as the extraction solvent. These extracts were examined against two gram-positive (Staphylococcus aureus, Bacillus cereus) and two gram-negative (E-coli, Salmonella Typhi) bacteria. Extracts prepared by all solvents showed antimicrobial action and established zone(s) of inhibition.

Aqueous extract

For the preparation of water extraction, a 10 g sample of Roselle was added to 100 ml distilled water, and the mixture was boiled for 10 min while stirring with a magnetic stirrer. Then, the extract was filtered from filter paper.

Alcoholic extraction

5g of powdered material along with 100 ml of alcohol was shaken well occasionally for the first 6 hr and kept undisturbed for 18 hr. The liquefied extract thus obtained was concentrated in a vacuum pump.

Phytochemical analysis

Extract and powder were checked for the presence of phytochemicals and respective phytochemicals are identified.

Test for Flavonoid

Extract of about 0.5 gm was dissolving in dilute NaOH and HCl was added. A yellow color solution turns colorless indicate the presence of flavonoid.

Test for Tannins

Extract of about 0.5 gm was mixed with water and heated on a water bath. The mixture was filter and Ferric Chloride solution was added to the filtrate. A dark–green solution shows the presence of tannins.

Antioxidant activity assay

The antioxidant activity analyzing using the DPPH and Ferric thiocyanate

Antioxidant activity assay DPPH Activity

DPPH radical scavenging activity

The free radical scavenging activity of Alcoholic and Aqueous Extract of Hibiscus sabdariffa Linn and ascorbic acid was measured in terms of hydrogen donating ability using the stable radical DPPH. About 0.3 mM solution of DPPH in 100% ethanol was prepared and 1 ml of this solution was added to 3 ml of the extract at different concentrations (10–100 mcg/ml). The mixture was shaken and allowed to stand at room temperature for 30 min and the absorbance was measured at 517 nm using a spectrophotometer. The IC50 value of the crude extract was compared with that of ascorbic acid, which was used as the standard. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity

Antioxidant activity assay Ferric thiocyanate

The antioxidant activity analysis using ferric thiocyanate was performed in the laboratory. 1 g of Roselle sample was dissolved in 0.20 ml of 98% ethanol, and 2.88 ml of a 2.51% linoleic acid solution in ethanol, and 10 ml of a 40 mM phosphate buffer (pH 7.0) were added. The mixture was incubated at 40°C in a stoppered test tube in the dark for 3 days. During the incubation, a 0.1 ml aliquot was taken from the mixture, and diluted with 9.7 ml of 75% ethanol, followed by the addition of 0.1 ml of 30% ammonium thiocyanate.3 min after adding the 0.1 ml of 20 mM ferrous chloride in 3.5% hydrochloric acid, the absorbance of the red color was measured at 500 nm.

Antimicrobial activity

The antimicrobial activity of the extracts was determined using the agar cup diffusion method. A 1 ml of an overnight culture of each bacterial isolate was added to agar plates maintained at 45°C. The pour plates were allowed to set, and a sterile cork borer of 8 mm diameter was used to cut equidistant wells on the surface of the agar. The wells were filled with 0.1 ml solution of each extract at a concentration of 10 mg/ ml. Penicillin at 5 μg ml-1 was added as the positive control. The plates were incubated at 37°C for 24 h after which the diameter of zones of inhibition was measured.

RESULT AND DISCUSSION

Preliminary phytochemical analysis showed the presence of flavonoid, tannins. Flavonoids and phenolic compounds have been reported to possess antioxidant activity. So the extracts were evaluated for in vitro antioxidant activity.

Over the years, the study on medicinal plants to show the mechanism of action and to justify their claims by traditional healers has been increased. An aim of this research has been the study of bioactive components and antioxidant properties of the H. sabdariffa L. The total phenolic compounds found in alcoholic and aqueous extracts of H. sabdariffa L. calyx were shown in figure no. 7. The figure shows that there is a rise in the amount of phenolic compound extracted by ethanol, which was (70.38 mg/g) than the amount of phenolic compound extracted by water which was (53.25 mg/g) at 5mg/ml.

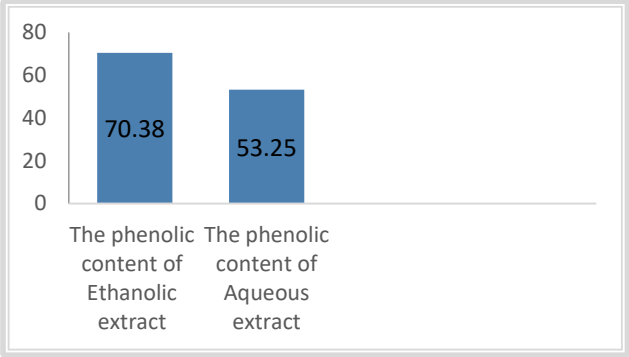

Fig.2: Total phenolic content of Roselle extracts.

DPPH radical scavenging activity

DPPH is nitrogen centered free radical that shows strong absorbance at 517 nm. Deep violet coloured methanolic DPPH solution changes to yellow colour in presence of DPPH radical scavengers. DPPH radical accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The extent of DPPH radical scavenged was determined by the decrease in the intensity of violet colour in the form of IC50 values. The antioxidant activity was compared with ascorbic acid (ASC) as standard. The Alcoholic extract shows the maximum antioxidant activity as compared to the aqueous activity.

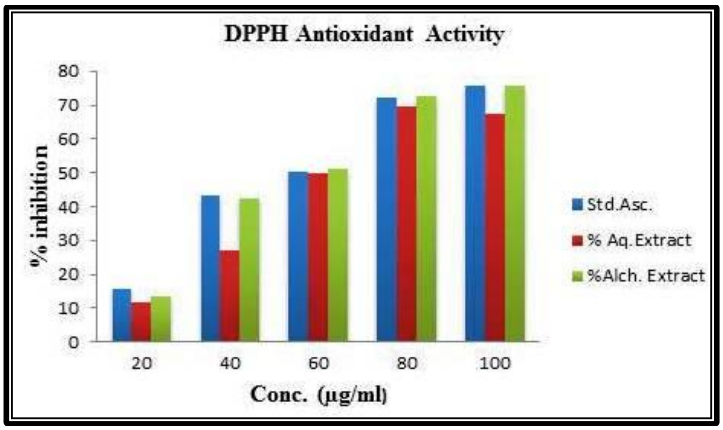

Fig.3: DPPH radical scavenging activity

Antioxidant activity assay Ferric thiocynante

The antioxidant activity was compared with ascorbic acid (ASC) as standard. The Alcholoic extract shows maximum antioxidant activity as compared to the aqueous activity.

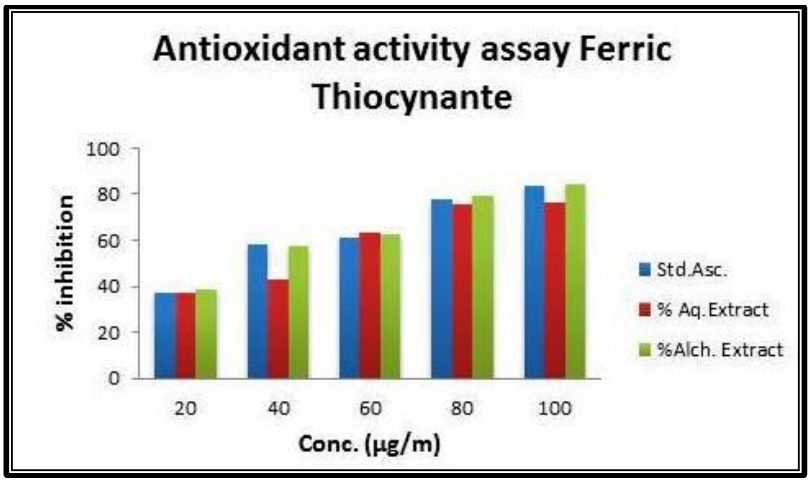

Fig.4: Antioxidant activity assay Ferric thiocynante

Antimicrobial Activity

Extract of H. Sabdariffa Linn. Shown zone of inhibition against Staphylococcus aureus, E.coli. Zone diameter is shown in Table 2. Staphylococcus aureus showed maximum zone of inhibition (Table No.2) Staphylococcus aureus causes nosocomial infection, blood infection, pimples, boils and other skin infections, scarlet fever, etc. It also acquired resistance to most antibiotics. Flower extract of H. Sabdariffa Linn also inhibits the growth of other disease-causing bacteria. This indicates that flower extract has the potential for treating infection caused by Staphylococcus aureus. Flower extract of Calendula officinalis shown zone of inhibition against Staphylococcus aureus, E.coli, Salmonella typhi. Zone diameter is shown in Table 2. E.coli shown maximum zone of inhibition by H.Sabdariffa Linn. Followed by Staphylococcus S .aureus.

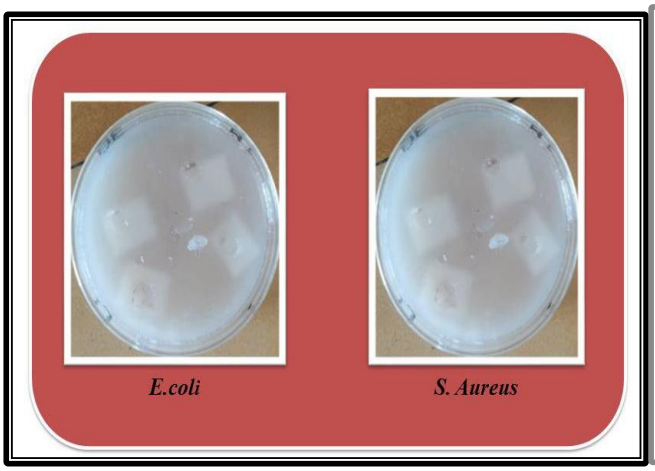

Fig.5: Zone of inhibition of E.coli and S. Aureus.

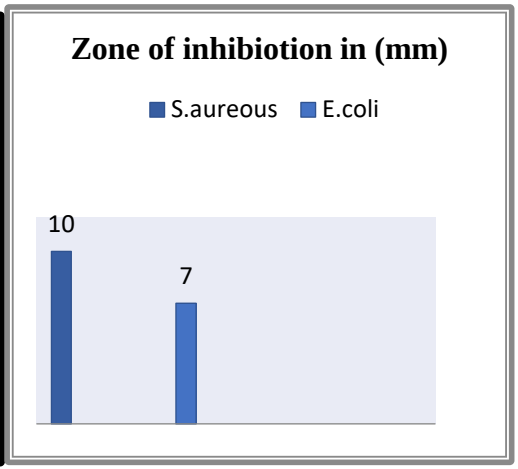

Fig.6: Antimicrobial effect of Hibiscus Sabdariffa Linn. extracts.

CONCLUSION

This present study concluded that plant extracts have antimicrobial and antioxidant potential. There are so many Roselle crop varieties developed, released, and used at the farmer's level for commercial cultivation. All those varieties of leaves and calyces have both vegetable and medicinal values. The fleshy red calyces are the most popular. The Roselle plant shows Antimicrobial activity as well as Antioxidant activity against E.coli, S.aureus. It shows greater antioxidant activity in ethanolic extract than aqueous extract.

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