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PHYTOCHEMISTRY, ANTI-OXIDANT, ANTI-INFLAMMATORY AND ANTI-MICROBIAL POTENTIAL OF SYZYGIUM SAMARANGENSE (ROSE APPLE) EXTRACT

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Abstract

This present investigation evaluated the phytochemistry, anti-oxidant, anti-inflammatory and anti-microbial potential of *Syzygium samarangense* (Rose Apple) extract. The methanol extract of fruit from *Syzygium samaranganse* was subjected to phytochemical analysis, which revealed the presence of various phytochemicals such as carbohydrates, tannins, saponins, alkaloids, flavonoids, quinones, and glycosides, with the exception of steroids, terpenoid, and phenols. In the DPPH test, the methanolic SSFE extract demonstrated a notable level of antioxidant properties. When comparison to the common medication Diclofenac, the methanolic SSFE displayed good anti-inflammatory properties using the Albumin denatured state method. The test against pathogenic bacterial strains involved various concentrations of methanolic SSFE (50, 100, and 150 mg/mL) and they all showed good activity.

Keywords: *Syzygium samarangense*, anti-oxidant, anti-inflammatory, anti-bacterial.

1. Introduction

The fruit of *Syzygium samarangense*, commonly known as the rose apple, has an oval to round shape with a rose-like scent [1,2,3]. *Syzygium samarangense* is rich in bioactive components, such as phenolic compounds, chalcones, flavonoids, flavanones, anthocyanosides, proanthocyanidins, and terpenoids [4,5,6]. These minute levels of bioactive compounds have numerous beneficial physiological and immunological impacts. They possess antioxidant, antibacterial, anticancer, and anthelmintic properties, among many other attributes. Phytochemicals possessing antibacterial properties, as reported by many researchers [7,8,9], damage cell membranes, impede the microbial metabolic process, and modify the signaling transduction pathway. Antioxidant phytochemicals reduce inflammation by blocking the formation of prostaglandins [10,11,12].

Plant-based medicines have been used extensively to treat a wide range of diseases since the beginning of human

civilization. Wax apple is the common name for Blume's *Syzygium samarangense*, a member of the Myrtaceae family. This extra-tropical tree is found mostly in Cambodia, India, Laos, Malaya, Thailand, and Vietnam. Typically, 5–15 m tall and 25–30 cm thick trees bear the pear-shaped fruit, which is 3.5–4 cm length and 4.5–5.4 centimeter wide has 4 fleshy calyxes and 0–2 spores. You can eat the fruits raw or cooked. Several components of *Syzygium samarangense* have been found to have potential medicinal benefits.

Several cytotoxic and antioxidant compounds were found in the extract of methanol of the pulp and seeds of *Syzygium samarangense.*. People with type II diabetes may benefit from fruit from *S. samarangense*. Antioxidants are substances that can stop and undo harm from too many free radicals. To enhance the quality of sleep and the functioning of the brain, *Averrhoa* are commonly used in alternative medicine. New research reveals that these effects may be caused by their special antioxidants. *Averrhoa* may improve immune function and inhibit the spread of cancerous cells. Reduced levels of free radicals and inflammation can aid in the prevention of chronic conditions like type 2 diabetes. The vitamin C-rich *Averrhoa* fruit is also known to have potent anticancer effects. *Averrhoa* fruit is delicious and tiny. They taste like dates when dried and have a chewy texture. Although loquats are frequently grown from seeds, commercial plantings typically use grafted trees of superior types.

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Shield budding and cleft grafting are the two methods used to reproduce the tree; loquat seedlings or quince rootstocks grown from cuttings can be used, the latter if a miniature tree is required [13,14,15].

The blossoms are relatively vulnerable to fire blight, but the trees are resistant to the majority of illnesses and insect pests. Hence, considering the above facts in view, this study evaluated the phytoconstituents of *Syzygium samarangense* fruit extracts with reference to their anti-oxidant, anti-inflammatory and anti-bacterial potential.

2. Experimental

2.1.Preparation of aqueous and solvent Syzygium samarangense fruit extracts:

The stored fruit powder of *Syzygium samarangense* (10 g) was extracted with 100 ml of methanol. After the extraction process, the solvents were removed by soxhlet method and evaporated by open air at 40°C to obtain crude extract and stored in beaker.

2.2. Phytochemical screening methanolic Syzygium samarangense fruit extracts:

Extracts Phytochemical screening of *Syzygium samarangense* fruit extracts were assessed by standard method as described by Gayathri and Jayaprakash (16).

Test for Tannins: One ml. of the fruit extract was added to 1 ml. 5% ferric Chloride Formation of dark blue or greenish black indicates the presence of tannins.

Test for Quinones: One ml. of the fruit extract was added to I ml. conc. Sulphuric acid. Formation of red colour indicates the presence of quinones.

Test for Flavonoids: One ml of the fruit extract was added to 1 ml. 2N sodium hydroxide. Formation of yellow colour indicates the presence of Flavonoids.

Test for Alkaloids: One mL of the fruit extract was added to 2 ml conc. HCI. Then, few drops of Mayer's reagent were added. Presence of green colour or white precipitate indicates the presence of alkaloids.

Test for Glycosides: One mL of the fruit extract was added to 3 mL Chloroform and 10% ammonium solution. Formation of pink colour indicates the presence of glycosides.

Test for Terpenoids: One mL of the fruit extract was added to 2 mL Chloroform along with cone, sulphuric acid. Formation of red brown Colour at the interface indicates the presence of terpenoids.

Test for Phenols: One mL of the fruit extract was added to 2 ml. distilled Water followed by few drops of 10% $FeCl_3$. Formation of blue/green colour indicates the presence of phenols.

Test for Steroids: One mL of the fruit extract was added to 2 ml. chloroform and 1 mL sulphuric acid. Formation of reddish brown ring at interface indicates the presence of steroids.

2.3.Antibacterial activity of methanolic Syzygium samarangense extracts:

Aqueous and solvent extracts of *Syzygium samarangense* fruit were tested against pathogenic bacterial strains namely *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli.* The bacterial cultures were grown in Mueller Hinton Agar and Broth (Himedia) (17). Antibacterial activity was measured using diffusion disc plates on agar, About 0.1 mL of each culture of bacteria was spread on agar plate surfaces. For antibacterial assay, all bacterial strains were grown in Mueller Hinton Broth (Hi media) for 24 hours at 37°C and plated on Mueller Hinton Agar (Hi media) for agar diffusion experiments. Paper discs (6 mm in diameter) were placed on the agar medium to load fruit extracts (100 µL) of *Syzygium samarangense*. Inhibition diameters were measured after incubation for 24 to 48 hours at 37°C. Blanks of solvent only (processed in the same way), were also tested for antibacterial activity.

2.4.Antioxidant activity of Syzygium samarangense fruit extracts:

The antioxidant activity of methanolic extract of *Syzygium samarangense* was determined by following and Shi *et al.* (18). About 100 µL of fruit extracts of *Syzygium samarangense* were taken in the microtiter plate, 100 µL of 0.1% methanolic DPPH was added over the samples and incubated for 30 minutes in dark condition. The samples were then observed for discoloration; from purple to yellow and pale pink were considered as strong and weak positive respectively. The antioxidant positive samples were subjected for further quantitative analysis.

2.4.1.Free radical scavenging activity of Syzygium samarangense fruit extract:

The antioxidant activities were determined using DPPH, (Sigma-Aldrich) as a free radical. Fruit extract of 100 µL were mixed with 2.7 mL methanol and then 200 µL of 0.1% methanolic DPPH was added. The suspension was incubated for 30 minutes in dark condition. Initially, absorption of blank sample containing the same amount of methanol and DPPH solution was prepared and measured as a control (16). Subsequently, at every 5 minutes

Table 1. Phytochemical screening of *Syzygium samarangense* fruit extract.

S.No.	Phytochemicals	Solvent (Methanol)
1.	Alkaloids	+
2.	Carbohydrates	+
3.	Flavonoids	+
4.	Glycosides	+
5.	Phenols	-
6.	Quinones	+
7.	Saponins	+
8.	Steroids	-
9.	Tannins	+
10.	Terpenoids	-

'+' – Present; '-'- Absent.

interval, the absorption maxima of the solution were measured using a UV double beam spectra scan at 517 nm. Free radical scavenging activity was calculated by the following formula:

% DPPH radical scavenging =[(Abs.of control-Abs.of test Sample)/(Abs. of control)] x 100.

2.5.Anti-inflammatory activity by egg albumin denaturation assay:

Inhibition of egg albumin denaturation was determined using the method prescribed by Chandra *et al.* (17). Phosphate buffer saline (pH 6.4), 8 g of sodium chloride (NaCl), 0.2 g of potassium chloride (KCl), 1.44 g of disodium hydrogen phosphate (Na_2HPO_4), and 0.24 g of potassium dihydrogen phosphate (KH_2PO_4) were dissolved in 800 ml of distilled water was prepared. The pH was adjusted to 6.4 using 1N hydrochloric acid (HCl) and made the volume to 1000 mL with distilled water. About 2.8 mL of phosphate buffer (pH 6.4) and 0.2 mL of egg albumin were incubated with various concentrations (10, 20, 30, 40 and 50 µg/mL) of test samples and standard drug Diclofenac sodium (10, 20, 30, 40 and 50 µg/mL) and the samples were incubated at 37°C for 15 minutes and heated at 70°C for 5 minutes. After cooling, the absorbance of the above solutions was measured using ultraviolet visible spectrophotometer at 660 nm. The percentage inhibition of protein denaturation was calculated using the following formula.

Percentage inhibition = (Abs control − Abs sample)/ Abs control × 100

3. Results and Discussion

3.1. Phytochemical screening of Syzygium samarangense fruit extract:

For the sake of human health, secondary metabolites provide essential medicinal qualities. In particular, some of these compounds appear to be capable of preventing and suppressing many types of cancer. Compounds belonging to the carbohydrate, alkaloids, quinones, and steroid families are utilized as medications or dietary supplements to treat or prevent various disorders. *Syzygium samarangense* fruit were gathered from Tirupattur for this investigation. The fruits were thoroughly cleaned in distilled water after being washed with running tap water, and they were then allowed to dry in the open air for about a month at room temperature. In order to be used later, the dried fruit material was thoroughly pulverized into powder and stored in a sterile container. *Syzygium samarangense* fruit powder that had been stored was extracted using 100 mL of each of the two solvents, methanol and chloroform. To get crude extract, the solvents were eliminated following the extraction procedure using air drying and an evaporator set at 40°C. *Syzygium samarangense* fruit extract phytochemical screening was evaluated using a conventional procedure as described by Gayathri and Jayaprakash (16). The methanol extract of fruit from *Syzygium samaranganse* was subjected to Phytochemical analysis, which revealed the presence of various phytochemicals such as carbohydrates, tannins, saponins, alkaloids, flavonoids, quinones, and glycosides, with the exception of steroids, terpenoid, and phenols. The phytochemical components of the methanolic extracts of Syzygium samaranganse are listed in Table 1.

3.2. Antibacterial activity of methanolic Syzygium samarangense fruit extract:

Throughout the beginning of human civilization, people have employed plants as medicine. The use of plants to treat diseases was inevitable, as is clear from the problems with synthetic antibiotics. Many researchers studied

Table 2. Antibacterial activity of methanolic *Syzygium samarangense* fruit extract.

S.No.	Organism	DMSO	Gentamycin	Zone of inhibition		
				50 µg/mL	100 µg/mL	150 µg/mL
1.	*Klebsiella pneumoniae*	-	33 mm	20 mm	21 mm	24 mm
2.	*Escherichia coli*	-	26 mm	18 mm	21 mm	23 mm
3.	*Staphylococcus aureus*	-	29 mm	17 mm	20 mm	22 mm
4.	*Enterococcus* sp.	-	34 mm	19 mm	23 mm	26 mm

Syzygium samarangense fruits using a variety of polar chemical solvents, including high (Methanol). Qualitative analysis and antimicrobial activity were examined. The greatest inhibitory zone was against *Enterococcus* sp., *Escherichia coli*, *Klebsiella pneumoniae*, and *Staphylococcus aureus* were 26 mm, 23 mm, 24 mm, and 22 mm when methanol extracts were compared to the well diffusion method. *Syzygium samarangense* that had been dissolved in DMSO (300 mg/mL) at doses of 50, 100, and 150 µg/mL had its antibacterial efficacy against the pathogens tested (Figure 1, Table 2).

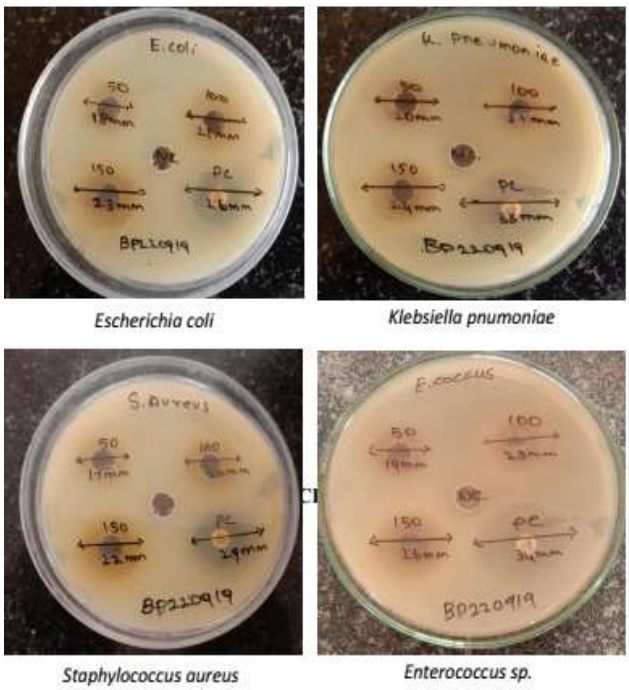

Figure 2. Antibacterial activity of methanolic *Syzygium samarangense* fruit extract

3.3. Antioxidant activity of Syzygium samarangense fruit extract:

Antioxidants are substances that can stop the chain reactions caused by free radicals. Recently, increased focus has been placed on the therapeutic potential of medicinal plants as antioxidants and re-antioxidants in avoiding tissue damage brought on by oxidative stress. It has been demonstrated that they can bind heavy metal ions, remove free radicals and active oxygen species, and stop lipid peroxidation by inhibiting lipoxygenase. Recently, increased focus has been placed on the therapeutic potential of medicinal plants as antioxidants and re-antioxidants in avoiding tissue damage brought on by oxidative stress. The antioxidant activity was assessed using the DPPH assay. The methanol extract of the *Syzygium samarangense* fruit was shown to have a higher level of free radical scavenging activity suggesting that it has a greater antioxidant potential. According to the DPPH assay, a decreased power potential was shown by an increase in absorbance with concentration. The methanol extract demonstrated strong reducing power used in the investigation; the reported inhibition percentage increased as the concentration increased (Figure 2).

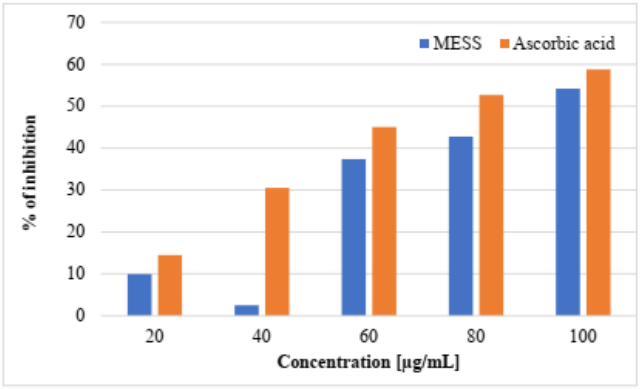

Fig. 2. Antioxidant activity *Syzygium samarangense* fruit extract.

3.4. Anti-inflammatory activity of Syzygium samarangense fruit extract:

The inhibition of egg albumin denaturation was assessed spectrophotometrically at 660 nm for *Syzygium samarangense* concentrations between 100-500 µg/mL, Diclofenac, and their interactions. *Syzygium samarangense* lowest dose of 10 g reduced denaturation of egg albumin protein. There was a progressively rising percentage of denaturation inhibition as *Syzygium samarangense* concentration increased. At various concentrations, diclofenac was found to have a denaturation-inhibiting effect on egg albumin, whereas *Syzygium samarangense* fruit had an identical impact (Figure 3).

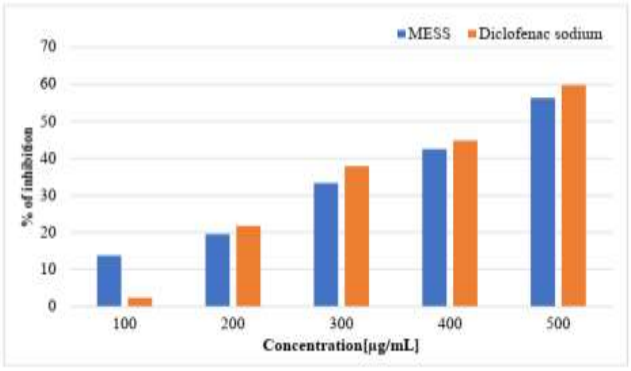

Fig. 3. Anti-inflammatory activity of *Syzygium samarangense fruit extract.*

4. Conclusion

The methanol extract of fruit from *Syzygium samaranganse* was subjected to phytochemical analysis, which revealed the presence of various phytochemicals such as carbohydrates, tannins, saponins, alkaloids, flavonoids,

quinones, and glycosides, with the exception of steroids, terpenoid, and phenols. In the DPPH test, the methanolic SSFE extract demonstrated a notable level of antioxidant properties. When comparison to the common medication Diclofenac, the methanolic SSFE displayed good anti-inflammatory properties using the Albumin denatured state method. The test against pathogenic bacterial strains involved various concentrations of methanolic SSFE (50, 100, and 150 mg/mL) and they all showed good activity.

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