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## *In vitro* antimicrobial activity and phytochemical analysis of fruits of *Syzygium aromaticum* (L.) Merr. & L.M.Perry - An important medicinal Plant

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### ABSTRACT

The fruit of *Syzygium aromaticum* is an important medicinal plant was subjected to phytochemical screening and antimicrobial effect of methanol, ethyl acetate and acetone extracts. Antimicrobial activity was analyzed by agar well diffusion method against gram positive and gram negative bacteria. The phytochemical analysis carried out revealed the presence of alkaloids, coumarins, catechins, flavanoids, phenols, saponins, terpenoids, tannins and steroids in various solvent extracts. Methanol extract of *S. aromaticum* showed good antimicrobial activity against *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. It has been showed that the methanol extracts had wider range of activity on these organisms than the acetone and ethyl acetate extracts, which indicates that the methanol extracts of selected plants may contain the active components. The use of plant extracts with known antimicrobial properties can be of great significance in therapeutic treatments.

**Keywords:** *Syzygium aromaticum*, Phytochemical analysis, Antimicrobial activity, Pathogens.

### INTRODUCTION

Plants produce a diverse range of bioactive molecules, making them rich source of different types of medicines. Most of the drugs today are obtained from natural sources or semi synthetic derivatives of natural products used in the traditional systems of medicine [1]. A wide range of medicinal plant parts are used for extract as raw drugs and possess varied medicinal properties [2, 3]. Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids [4, 5]. The secondary compounds include alkaloids, flavonoids, terpenoids, steroids, anthraquinones and volatile oils [6]. Phytochemical analysis revealed that phenolic compounds especially alkaloids are responsible for the antimicrobial activity. Two types of antifungal compounds exist in the plants. The compounds, which occur naturally in high concentrations, are the constitutive compounds and those produced in response to fungal infections are known as phytoalexins. The role of these compounds in plant resistance against fungi has been reported [7]. The antimicrobial potential of different medicinal plants are being extensively studied all over the world, but only a few studies have been systematic manner [8]. The present study was aimed to evaluate the phytochemical screening and antimicrobial activity of *Syzygium aromaticum* fruit extracts.

### MATERIALS AND METHODS

#### Collection of plant material

The fresh plants materials were collected from Courtallum hills, Tirunelveli District, Tamil Nadu, and India. Taxonomic identification of the plants was carried out with the help of Dr. R. Ramasubbu Assistant professor, Ganghigram Rural Institute Dindigul, Tamil Nadu, and India.

#### Preparation of fruit extracts

The dried fruit extracts were prepared by sequential extraction method using three organic solvents in the basis of polarity of solvents (Methanol, Ethyl acetate and Acetone). 25g of the fresh fruit sample was taken in a conical flask and 200 ml of Acetone was added. The conical flask was kept on mechanical shaker for 24 hours, after that the extract was filtered through Whatman filter paper 1 and the pellet was allowed for drying and this pellet was used for the next solvent extraction (Ethyl acetate and Methanol). The dried extract was recovered and stored in refrigerator for further analysis.

### Extract recovery percentage

The percentage of extracts yield was calculated using the following equation.

$$\% \text{Yield} = \frac{\text{Extract + container (g)} - \text{Empty container (g)}}{\text{Sample weight (g)}} \times 100$$

### Extracts screening

The dried fruit extracts were subjected to antimicrobial activity and qualitative phytochemical screening for the identification of various classes of active biochemical constituents.

#### Test for Alkaloids (Mayers Test)

To 1 ml of fruit extracts, 6 drops of Mayers reagent was added. The formation of yellowish creamish precipitate indicated the presence of alkaloids [9, 10].

#### Test for Saponins (Foam Test)

1ml of fruit extracts were mixed with 5ml of distilled water. The contents were heated in a boiling water bath. Frothing indicated the presence of saponins [9, 10].

#### Test for Tannins (Braymer's Test)

1ml of the fruits extracts were mixed with 2ml of water. To this, 2 drops of 5% ferric chloride solution was added. Appearance of dirty green precipitate indicated the presence of tannins [9, 10].

#### Test for steroids (Salkowski Test)

To 2ml of the fruit extracts, 2ml of chloroform was added followed by concentrated sulphuric acid. Formation of reddish brown ring at the junction showed the presence of steroids [11].

#### Test for terpenoids

2ml of the fruit extracts were treated with 2ml acetic acid. Then concentrated sulphuric acid was added. Deep red color development showed the presence of terpenoids [11].

#### Test for Coumarins

2ml of the extracts were taken and 3ml of 10% sodium hydroxide was added. Formation of yellow coloration indicated the presence of coumarins [11].

#### Test for Catechins

2ml of alcoholic fruit extracts solution were treated with few drops of Echrilich reagent and few drops of concentrated HCL. The pink color formation indicated the presence of catechins [11].

#### Test for phenols

1ml of the fruit extracts were treated with 3% ferric chloride. The appearance of deep blue color shows the presence of phenols [12, 13].

#### Test for flavonoids

1ml of the fruit extracts were added to 1ml of sulphuric acid. Orange color formation confirmed the presence of flavonoids [12, 13].

### Test for Quinones

1ml of the fruit extracts were treated with 5ml of HCL. Formation of yellow color precipitate indicated the presence of quinones [12, 13].

### Antimicrobial activity

*Staphylococcus aureus*, *Streptococcus faecalis*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and fungus *Candida albicans*. All samples were kindly donated by the Department of Biology Gandhigram Rural Institute, Dindugul.

The test organisms were maintained on nutrient agar slant and kept in a refrigerator at 4°C. 100ml aliquots of nutrient broth were inoculated with the culture of test micro-organisms using a loop and then incubated at 37°C for 24 hrs.

Antimicrobial activities of methanol, ethyl acetate and acetone fractions of plants were carried out using the agar well diffusion method. Mueller-Hinton agar medium (MHA) was used for antimicrobial susceptibility tests. The MHA medium was prepared by pouring 20 ml of molten media into sterile Petri plates. The plates were allowed to solidify and 0.2ml of an overnight broth culture of test micro-organisms was added to 20ml of cooled molten agar on the medium and allowed to dry for 5 min. For agar well diffusion method, four equidistant wells (6 mm in diameter) were cut from the agar with the help of a cork-borer. 40 µl of fruits extracts (methanol, ethyl acetate and acetone extracts) containing (4 mg) concentration was loaded on 6 mm well. The standard antibiotic solution Gentamicin (10 µg) was placed on the surface of the plates. The plates were kept for incubation for 24 hrs at 37°C. The zone of inhibition was measured around the well containing samples and standard. The experiments were performed in triplicates.

### Statistical Analysis

All the data was reported as mean ± standard deviation of three replicates. Statistical analysis was performed using Microsoft Excel.

## RESULTS AND DISCUSSION

Extraction methods used pharmaceutically involves the separation of medicinally active portions of plant tissues from the inactive/inert components by using selective solvents. During extraction, solvents diffuse into the solid plant material and solubilize compounds with similar polarity [14]. The extractive values of three different solvents extracts are given in Table 1. The highest extractive yield was found in the methanol extract.

**Table 1:** Extractive value of plant extracts

Plant extracts	Yield % (W/W)
Methanol	10.8%
Ethyl acetate	8.5%
Acetone	7.46%

Preliminary qualitative test according to [15] is useful in the detection of bioactive principles and subsequently may lead to drug discovery and development [16]. Table 2 shows all the three extracts possessed the phytochemical constituents such as flavonoids and phenols. Methanol and ethyl acetate extracts showed the presence of flavonoids, phenolics, tannins, terpenoids and steroids while alkaloids, coumarins and quinones were absent. But catechins only present in the methanol extract. On the other hand, acetone extract showed the presence of coumarins, phenols, flavonoids and alkaloids, while saponins, tannins, terpenoids, quinones and steroids were absent.

Many efforts have been made to discover new antimicrobial compounds from various kinds of sources such as micro-organisms,

animals, and plants [17]. Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the *in vitro* antibacterial activity assay [18]. The results of antimicrobial screening as measured by diameter of zone of inhibition are shown in Table 3. In *Syzygium aromaticum* methanolic fruit extract, the antimicrobial activity ranged from (8.5±0.50 mm) to (13.2±0.29 mm). The methanolic extracts of

activity were highly against *Pseudomonas aeruginosa* (13.2±0.29 mm) and least activity of ethyl acetate extracts against *Bacillus subtilis*. The acetone extract also exhibited significant activity. Similarly, the acetone extract was found to be active with the zone of inhibition ranges between (9.8±0.76 mm) to (11.9±0.81mm). They ethyl acetate extracts no responsible against *Escherichia coli* (0.00±0.00 mm).

**Table 2:** Preliminary phytochemical screening of different extract of *Syzygium aromaticum* fruits

Phytochemical tests	Different solvent extracts		
	Methanol	Ethyl acetate	Acetone
Alkaloids	-	-	+
Flavonoids	+	+	+
Phenols	+	+	+
Saponins	+	-	-
Tannins	+	+	-
Terpenoids	+	+	-
Quinones	-	-	-
Catechins	+	-	-
Coumarins	-	-	+
Steroids	+	+	-

+ = indicates presence of phytochemicals, - = indicates absence of phytochemicals.

**Table 3:** Antimicrobial activity of three solvent extracts from the fruits of *Syzygium aromaticum*

Microbial strain	Solvent Extracts (Zone of inhibition in mm)			
	Methanol	Ethyl acetate	Acetone	Gentamicin
<i>Escherichia coli</i>	8.5±0.50	0.0±0.00	9.8±0.76	20.0±1.00
<i>Staphylococcus aureus</i>	11 ±0.00	10.5±0.58	11.8±0.72	22.1±0.23
<i>Streptococcus faecalis</i>	9.2±0.29	9.3±0.58	10.7±0.58	21.9±0.23
<i>Pseudomonas aeruginosa</i>	13.2±0.29	10.3±0.58	11.9±0.81	21.2±0.29
<i>Candida albicans</i>	8.7±0.58	10.5±0.50	9.8±0.76	20.9±0.81
<i>Klebsiella pneumoniae</i>	11.2±0.9	9.3±0.58	10.5±0.50	19.3±0.50
<i>Bacillus subtilis</i>	12.5±0.50	8.5±0.50	10.5±0.50	23.5±0.58

Each value represents Mean ± SD of three replicates

In previous reports of *Syzygium aromaticum* flower extract revealed highest antimicrobial activity against tested microbial pathogens [19]. Earlier finding of data supported to our results of *Syzygium aromaticum* fruit extracts activity. There is no any data published on the antimicrobial activity of *Syzygium aromaticum* fruit of different solvent extracts. Some antibacterial activity of *S. aromaticum* fruit extracts against *staphylococcus aureus* and *Bacillus cereus* has been established, but no antifungal activity [20].

**CONCLUSION**

The results of present work established that all the extracts possess number of important phytocostituents and antimicrobial activity against selected microbes. The results provide justification for the use of these plants in folk medicine to treat various infectious diseases. Further, can be investigation for the development of new potential antimicrobial compounds.

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