

**VOLUME 9**

**ISSUE 2**

**2023**

**ISSN 2454 – 3055**



**INTERNATIONAL  
JOURNAL OF  
ZOOLOGICAL  
INVESTIGATIONS**

***Forum for Biological and  
Environmental Sciences***

**Published by Saran Publications, India**



## **Phytochemical Analysis and Antimicrobial activity of the Plant *Syzygium aqueum***

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**Received:** 20<sup>th</sup> May, 2023; **Accepted:** 12<sup>th</sup> July, 2023; **Published online:** 19<sup>th</sup> August, 2023

<https://doi.org/10.33745/ijzi.2023.v09i02.037>

**Abstract:** *Syzygium aqueum* belongs to the family Myrtaceae. This study was aimed to evaluate antimicrobial activity of *Syzygium aqueum* and the presence of primary and secondary metabolites. Leaves of the plant *Syzygium aqueum* were collected and subjected to phytochemical analysis. The antimicrobial activity of leaf extract of *Syzygium aqueum* was tested against Gram positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*, *Viridians streptococci* and *Streptococcus pyogenes*) and Gram negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Klebsiella pneumonia*). The screening of bioactive components indicates the presence of Flavonoids, Tannins, Glycerides, Steroids, Terpenoids, Phenols, Carbohydrates, Proteins and amino acids. Crude ethanolic extract of leaves of *Syzygium aqueum* is effective against the growth of bacteria.

**Keywords:** *Syzygium aqueum*, Phytochemical, Gram positive bacteria, Gram negative bacteria, Antimicrobial analysis, Zone of inhibition

**Citation:** Davis Dessy and Jose Sajini: Phytochemical analysis and antimicrobial activity of the plant *Syzygium aqueum*. Intern. J. Zool. Invest. 9(2): 345-350, 2023.

<https://doi.org/10.33745/ijzi.2023.v09i02.037>



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### **Introduction**

Natural resources are those that come from living things like plants, animals, and microorganisms. Phytochemicals are the substances synthesized by plants (Rehmanav *et al.*, 2020). Plants' phytochemicals have long been a source of medical treatments for human illnesses. According to the World Health Organization, these are essential to the primary healthcare provided to about 75–80 per cent of the world's population. Many techniques, including extraction, separation,

purification, identification, structural elucidation, assessment of physical and chemical characteristics, biosynthesis, and quantification, can be used to investigate phytochemicals in a plant (Anulika *et al.*, 2016).

Both primary and secondary metabolites could be used to describe the phytochemicals. Natural sugars, amino acids, proteins, purines, pyrimidines of nucleic acids, and chlorophyll are among the primary metabolites. The other plant compounds,

including glycosides, alkaloids, terpenoids, flavonoids, lignans, steroids, curcumines, saponins, and phenolics, are classified as secondary metabolites (Saxena *et al.*, 2013).

*Syzygium* is the 16<sup>th</sup> biggest genus of flowering plants in the Myrtaceae family (Nair *et al.*, 2017) and is widely farmed for a variety of uses, including the production of colourful, palatable fruits (Wardana *et al.*, 2018).

Several studies have discussed plentiful supplies of phytochemical components and biological activity. The bioactivities and phytochemical components of *Syzygium aqueum*, however, have not yet been reported. Therefore this study was aimed to provide the phytochemical analysis and antibacterial activities of the plant *Syzygium aqueum*.

## Materials and Methods

### Plant collection:

The leaves of *Syzygium aqueum* were collected from in and around Kerala. Collected leaves were washed 2-3 times in running tap water followed by sterilized distilled water and were dried in shade for 20-25 days. Dried leaves were separately crushed and ground into fine powder using electric blender. Plant specimen were identified, certified and the voucher specimen was deposited at Botanical Survey of India.

### Plant extraction Method:

The leaves were washed with copious amounts of water and distilled water. These were then allowed to air dry at room temperature for 2-3 h. Following this the leaves were placed in a circulating oven at 40 °C until completely dried. The dried plants were powdered using a Fritsch dry miller. Ethanol (analytical grade) at 1: 10 (w/v) concentrations was added to the powdered leaves. Ethanol extraction was carried out at room temperature and was left for 24 h in an orbital shaker and Methanol at 1:10 (w/v) concentrations was added to the powdered leaves. Methanolic extraction was carried out at room temperature and was left over shaking for 24 hrs

in an orbital shaker. The suspension obtained was filtered using a 114bWhatman filter paper and the filtrate collected. The ethanol filtrate was concentrated using evaporator at 40 °C.

### Phytochemical screening:

#### Steroids:

**Salkowski test:** 1 ml of the extract was treated with 3-4 drops of concentrated sulphuric acid. The lower layer turned red in color showing the presence of steroids.

#### Alkaloids:

**Wagner's Test:** 1 ml of the extract was treated with 3-5 drops of Wagner's reagent. The formation of reddish brown precipitate showed the presence of alkaloids.

#### Flavonoids:

**Ferric Chloride Test:** 1 ml of methanol extract was mixed with few drops of ferric chloride, formation of blackish red precipitate showed the presence of flavonoids.

#### Phenolic Compounds:

**Ferric Chloride Test:** 3 – 4 drops of ferric chloride were added to 1 ml of extract, the solution appeared blue colour indicating the presence of Hydrolysable tannins.

#### Glycosides

**Keller – Killani Test:** 2 ml glacial acetic acid containing a drop of FeCl<sub>3</sub> was added to plant extract. Formation of a brown color ring indicated the presence of glycosides.

#### Triterpenoids:

**Salkowski Test:** The extract was mixed with 2 ml chloroform and few drops of conc. Sulphuric acid and shaken well. Formation of reddish-brown colour at lower layer indicated presence of steroids and yellow colour showed the presence of triterpenoids.

#### Protein and Amino acid:

**Million's Test:** 2 ml test solution was added with

Millions precipitate, which on heating turned to red.

**Ninhydrin Test:** 2 ml test extract was mixed with 0.2 % ninhydrin solution and boiled. Formation of blue colour indicated the presence of protein.

#### **Carbohydrates:**

**Molish test:** Firstly 2 ml extract was placed in a test tube then 1 drop of Molish Reagent was added. 2 ml of concentrated HCl was added from the sides of the test tube. Formation of a violet ring at the junction of the two liquids indicated presence of carbohydrates.

#### **Saponins:**

**Foam Test:** 0.5 g of extract was shaken with 2 ml of water then heated to boil. Frothing showed the presence of saponins.

#### **Anthraquinones:**

5 ml of the extract solution was hydrolysed with diluted conc. H<sub>2</sub>SO<sub>4</sub> and extracted with benzene. 1 ml of dilute ammonia was added to it. Rose pink colouration suggested the positive response for anthraquinones.

#### **Antibacterial activity:**

Eight bacterial strains namely, *Escherichia coli*, *Klebsiella pneumonia*, *Proteus mirabilis*, *Staphylococcus aureus*, *Viridans streptococci*, *Streptococcus pyogenes*, *Bacillus subtilis* and *Pseudomonas aeruginosa* were clinically isolated and were used for antimicrobial testing. All bacteria were purchased from P.S.G Institute of Medical Sciences and Research. Broth cultures of the pure culture isolates of these test microorganisms used in the present study were prepared by transferring a loop of culture into sterile nutrient broth and incubated at 37 °C for 24 h. A loop full was taken from these broths and seeded onto sterile nutrient agar plates through sterile cotton swab to develop diffused heavy lawn culture. The well was prepared with help of sterile 6 mm diameter pen cork. Then 100 µl of prepared leaf extracts of *Syzygium aqueum* were tested by agar well diffusion method to determine the

antimicrobial activity using standard procedure. 1000 ppm concentrations of extract were used to perform this study. The well were placed with 10 ml antibiotic (ampicillin) as control. In the treatment the seeded bacterial plates wells were treated with 25 ml extracts. The plates were then incubated at 37 °C for 24 h and then examined for clear zones of inhibition around the wells impregnated with particular concentrations of drug.

## **Results**

#### **Phytochemical Screening:**

Qualitative phytochemical analysis of Methanol and Ethyl acetate leaf extract of *Syzygium aqueum* were tested for the presence of few primary and secondary metabolites. DPPH were used to determine the antioxidant potential of the selected extracts.

The results of phytochemical analysis revealed that both extracts tested contain the secondary metabolites, the Methanolic leaf extract of *Syzygium aqueum* revealed the presence of flavonoids, phenolic compounds, glycosides, tannins, triterpenoids, carbohydrates, protein and amino acids whereas anthraquinones, alkaloids, saponins and steroids were absent (Table 1).

In ethyl acetate extract flavonoids, phenolic compounds, glycosides, tannins, triterpenoids, carbohydrates, protein and amino acids showed the presence and the absence of anthraquinones, alkaloids, saponins and steroids.

#### **Antimicrobial Activity:**

Using Methanol and Ethyl acetate as the extraction solvents, *Syzygium aqueum* leaf extract showed good inhibitory activity against Gram positive and Gram negative human pathogens. Using Methanol and Ethyl acetate solvents, the antibacterial activity was only performed at 1000 ppm concentration in all crude extracts from *Syzygium aqueum* leaves. The inhibitory effect of leaf extract of *Syzygium aqueum* were tested against diverse pathogens. Ampicillin 10 mg was the standard antibiotic that was employed. This

Table 1: The phytochemical analysis of various leaf extract of *Syzygium aqueum*

| S. No. | COMPOUNDS                | METHANOL | ETHYL ACETATE |
|--------|--------------------------|----------|---------------|
| 1      | Alkaloids                | -        | -             |
| 2      | Flavonoids               | +        | +             |
| 3      | Anthraquinones           | -        | -             |
| 4      | Tannins                  | +        | +             |
| 5      | Saponins                 | -        | -             |
| 6      | Glycerides               | +        | +             |
| 7      | Steroids                 | -        | -             |
| 8      | Terpenoids               | +        | +             |
| 9      | Phenols                  | +        | +             |
| 10     | Carbohydrates            | +        | +             |
| 11     | Proteins And Amino Acids | +        | +             |

Table 2: Antibacterial activity of Methanol extracts of *Syzygium aqueum* against Gram positive and Gram negative bacteria at 1000 ppm concentrations

| ORGANISM                      | ZONE OF INHIBITION ( mm) |                               |
|-------------------------------|--------------------------|-------------------------------|
|                               | Zone of inhibition       | Positive control (Ampicillin) |
| <i>Escherichia coli</i>       | 5.75 ± 1.70              | 10.75 ± 2.21                  |
| <i>Pseudomonas aeruginosa</i> | 5.5 ± 0.57               | 17 ± 2.44                     |
| <i>Proteus mirabilis</i>      | 6 ± 0                    | 13.5 ± 2.64                   |
| <i>Staphylococcus aureus</i>  | 8.5 ± 1.91               | 12.25 ± 2.98                  |
| <i>Klebsiella pneumonia</i>   | 6.5 ± 1.29               | 14 ± 1.15                     |
| <i>Bacillus subtilis</i>      | 5 ± 0.81                 | 15 ± 1.82                     |
| <i>Streptococcus pyogenes</i> | 13.75 ± 3.20             | 8 ± 0.81                      |
| <i>Viridans streptococci</i>  | 6 ± 0.81                 | 8 ± 0.81                      |

study included Gram positive (*Staphylococcus aureus*, *Bacillus subtilis*, *Viridans streptococci* and *Streptococcus pyogenes*) and Gram negative (*Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Klebsiella pneumonia*) bacteria. The methanolic plant extract is active significantly against majority of the chosen microorganisms.

The zone of inhibition in Methanol extract (Table 2) showed by *Escherichia coli* is 5.75 ± 1.70 mm where as 5.5 ± 0.57 mm, 6 ± 0 mm, 8.5 ± 1.91 mm, 6.5 ± 1.29 mm, 5 ± 0.81 mm, 13.75 ± 3.20 mm and 6 ± 0.81 mm were showed by *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Bacillus subtilis*,

Table 3: Antibacterial activity of Ethyl acetate extract of *Syzygium aqueum* against Gram positive and Gram negative bacteria at 1000 ppm concentrations.

| ORGANISM                      | ZONE OF INHIBITION ( mm) |                              |
|-------------------------------|--------------------------|------------------------------|
|                               | Zone of inhibition       | Positive control(Ampicillin) |
| <i>Escherichia coli</i>       | 10.5 ± 1.91              | 10.75 ± 2.21                 |
| <i>Pseudomonas aeruginosa</i> | 7.5 ± 1.29               | 17 ± 2.44                    |
| <i>Proteus mirabilis</i>      | 8.75 ± 2.36              | 13.5 ± 2.64                  |
| <i>Staphylococcus aureus</i>  | 9 ± 1.63                 | 12.25 ± 2.98                 |
| <i>Klebsiella pneumonia</i>   | 11.75 ± 2.5              | 14 ± 1.15                    |
| <i>Bacillus subtilis</i>      | 6.25 ± 3.20              | 15 ± 1.82                    |
| <i>Streptococcus pyogenes</i> | 9.5 ± 4.35               | 8 ± 0.81                     |
| <i>Viridans streptococci</i>  | 7.5 ± 3.10               | 8 ± 0.81                     |

*Streptococcus pyogene* and *Viridans streptococci*, respectively.

Likewise in Ethyl acetate extract of *Syzygium aqueum* (Table 3), zone of inhibition for *Esherichia coli* is 10.5 ± 1.91 mm, whereas *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Bacillus subtilis*, *Streptococcus pyogene* and *Viridans streptococci* showed 7.5 ± 1.29 mm, 8.75 ± 2.36 mm, 9 ± 1.63 mm, 11.75 ± 2.5 mm, 6.25 ± 3.20 mm, 9.5 ± 4.35 mm and 7.5 ± 3.10 mm zone of inhibition, respectively.

Zone of inhibition ranged between 6.25 ± 3.20 mm to 11.75 ± 2.50 mm in methanol and 5.00 ± 0.81 mm to 13.75 ± 3.20 mm in Ethyl acetate extract of *Syzygium aqueum*. Ampicillin showed a significant superiority in the zone of inhibition as compared to the test extracts.

The stronger antibacterial activity with maximum zone of inhibition 13.75 ± 3.20 mm was recorded with Ethyl acetate extract of *Syzygium*

*aqueum* against *Streptococcus pyogenes* at 1000 ppm concentration and minimum zone of inhibition 5.00 ± 0.81 mm was recorded with ethyl acetate of *Syzygium aqueum* against *Bacillus subtilis* at 1000 ppm concentration.

## Discussion

Many phytochemicals including alkaloids, cyanogenic glycosides, phenyl-propanoids, polyketides, anthocyanins, carbohydrates, amino acids, terpenoids, flavonoids, phenols, saponins and tannins are present in the majority of plant species (Nxumalo *et al.*, 2021). Phytochemical screening was performed to identify and ensure that these plants contain compounds that perform bioactivities, such as antioxidant and cytotoxic compounds. Phytochemical studies show that the leaves of both variations of *Syzygium aqueum* contained the same compounds, namely phenolics, flavonoids and terpenoids (Afrizal *et al.*, 2021). In the present phytochemical study, *Syzygium aqueum* leaf extract tested positive for alkaloids,



glycosides, flavonoids, tannins, triterpenoids, phenols, carbohydrates, saponins, phytosterols, proteins and amino acids. The above results are in conformity with the findings of Feroz *et al.* (1993).

The antimicrobial activity of *Syzygium aqueum* leaves extract for the selected bacteria was assessed using agar well diffusion method by measuring the diameter of growth inhibition zones. These results showed that the extract had inhibition zones effect on the growth of bacterial species. The antimicrobial activity corresponds to the concentration of the extract, where the mean zone of inhibition decreases when the concentration is low (Habisukan *et al.*, 2021).

## Conclusion

Many parts of the world have used *Syzygium aqueum* in traditional medicine. Studies have verified the presence of numerous bioactive chemicals and various pharmacological actions, even though many of them have not yet been measured. Research is needed to identify the numerous novel secondary metabolites because of the plant's phytotherapeutic value. The plant has a lot of potential as a long-used medicinal plant, but that potential has not been fully realized yet. Although *Syzygium aqueum* is frequently discussed, no clinical trials have been conducted on it. So, in the near future, clinical evaluation should serve as a baseline for this species safe medicinal applications. The basic studies on *Syzygium aqueum* in Methanol and Ethyl acetate have been explored in this paper, which will be useful to researchers for conducting additional research on this plant.

## Acknowledgements

Thanks to the Head, Department of Zoology, Nirmala College for Women for the help during this study.

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