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# EVALUATION OF PHYTOCHEMICAL CONSTITUENTS AND ANTIBACTERIAL ACTIVITY IN LEAF EXTRACTS OF SYZYGIUM CUMINI L

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## ABSTRACT

Jambolan (*Syzygium Cumini* L) Belongs to the family *Myrtaceae* is one of the widely used medicinal plants in the treatment of various diseases in particular diabetes. The present investigation has been primed to describe the phytochemical constituents of leaf extracts of *S.cumini* L. (Jambolan), Jamun is a large evergreen beautiful tree of Indian subcontinent. The leaves of *Syzygium cumin* L is considered as an antibacterial and also used to strengthen the teeth and gums in folklore medicine. In the present investigation different tests were conducted for the presence of various phytochemical compounds in *Syzygium cumini L* leaf extracts. Glycosides, Tannins and Phenols were commonly found in ethanol and methanol extracts of leaves that were not seen in the extracts of chloroform and pet ether. Among all the Extracts tested methanolic extracts showed more phytochemicals than the other extracts. Phytochemicals like amino acids, terpenoids and cardioglycosides were not found in leaf methanol, ethanol, chloroform, hot water and pet ether extracts. The analysis revealed activity of leaf extracts of *Syzygium cumini* L against the bacteria in the order *Salmonella typhi, E. coli, Streptococci, Bacillus cereus, Pseudomonas aeruginosa and Bacillus subtilis*. All extracts showed maximum activity on *Salmonella typhi* and *E. coli*. Ethanol, methanol and hot water extracts showed more inhibition zones against bacteria than the extracts of chloroform and petroleum ether. Results indicated dosage reliant antibacterial activity of leaf extracts of Syzygium *cumini L*.

**KEYWORDS:** Phytochemicals, leaf extracts, *Syzygium cumini L*.

## INTRODUCTION

About 25% of globally prescribed drugs obtained from plants are used as natural products in diseases prevention and control as well as in drug development. Syzygium cumini popularly known as "jamolao" belonging to myrtaceae family is one of the most commonly used medicinal plant. The use of natural products in diseases prevention and control as well as in drug development about 25% of globally prescribed drugs obtained from plant. It has also been very well documented in the world forum WHO's report which state that more than 80% of world's population are dependent on plants to meet their primary health care needs (Ahmadullah and Nagar, 1999) . Plant metabolites have been of great interest to man for long time due to their pharmacological relevance (Arora, kaur &kaur, 2013). A large proportion of world population, especially in the developing countries depends on traditional system of medicine for many diseases. Plant based drugs constitute a major share of medecine in India, china viz ayurveda, yoga, unani, siddha, homeopathy and naturopathy, except allopathy (Vaidya & Pevasagagam, 2007).

S. cumini has been widely used for the treatment of various diseases in traditional and folk medicine. Unani system of medicine describes the use of this plant as liver tonic, enrich blood, strengthening teeth and gums and also form good lotion for removing ringworm (Ayyanar et al., 2012). The entire plant parts such as leaves, flowers, bark fruit, seeds of have been reported to be used in medicine *Syzygium cumini*. Charaka used seeds, leaves and fruits in decoction for diarrhoae and the bark as an astringent. The leaves have antibacterial property and used to strengthen the teeth and gums. The leaves have also been extensively used to treat diabetes, constipation, leucorrhoea, stomachalgia, fever, gastropathy, dermopathy and to inhibit blood discharge in the feces (Ravi 2005, Sagrawat *et al*., 2006, Gowri and Vasantha, 2010).

The bark, leaves and seed extracts of *s.cumini* have also been reported to possess anti-inflammatory, antibacterial and antidiarrheal effects (Indira and Mohan, 1992). The major phytoconstituents are reported to contain anthocyanins (Wealth of India, 1976). Preliminary phytochemical analysis also showed the presence of phenols, terpenoids, tannins, saponins, phytosterols, carbohydrates, flavonoids, amines in stem bark of *syzygium cumini*. The present study was designed to investigate the phytochemical bioactive compound and antibacterial activity of *syzygium cumini* leaf methanolic extracts. The leaves have been extensively used to treat diabetes, constipation (Bhandary et al., 1995), leucorrhoea, stomachalgia, fever,

gastropathy, strangury and dermopathy (Warrier et al., 1996), and to inhibit blood discharges in the faeces (Bhandary et al., 1995). The plant possesses acetyl oleanolic acid, triterpenoids, ellagic acid, isoquercitin, quercetin, kaempferol and myricetin in different concentrations (Rastogi and Mehrotra, 1990). Most of these compounds have been reported to possess antioxidant and free radical scavenging activities (Tanaka et al., 1998).

## MATERIALS AND METHODS

Fresh plant leaves of *Syzygium cumini* was collected from University College of science, Saifabad, Hyderabad in March 2013. The plant materials they collected were washed with tap water and shade dried at room temperature for one week then they were ground to fine powder with the help of pastel and mortar and were used for further studies.

### Preparation of Crude Extracts

Ten grams of dried leaves powder of *syzygium cumini* subjected to rotar evaporator. All the extracts were stored in the refrigerator at 4 degree centigrade for future use. Different tests conducted for the identification of phytochemicals is adopted by using the methods described by (Edeogal *et al*., 1996) (Thammilmalai selvi *et al*., 2013) (Jyothi chaitanya and Lakshmi Bhavani, 2013). To the 5ml of extract 5ml of 2N Hcl is added and boiled and then the mixture is filtered. To the filtrate a few drops of Mayer's reagent is added cream color precipitate was produced immediately indicating the presence of alkaloids.

Saponins are tested by boiling 5ml of extract in 10ml of distilled water in a test tube and are shaken vigorously for about 30 seconds. The test tube is allowed to settle for half an hour. Formation of froth indicates the presence of saponins.

Tannins are tested by adding a few drops of 1% lead acetate to 5 ml of plant extract. Appearance of yellow precipitate indicates the presence of tannins.

Phenols are tested by adding 2ml of ferric chloride solution to 2ml of plant extract. Appearance of bluish green colour solution indicates the presence of phenols.

For testing the presence of steroids 1ml extract was dissolved in 10ml of chloroform and equal volume of concentrated sulphuric acid was added from the walls of the test tube. Appearance of red colour in the upper layer and yellow with green fluorescence indicates the presence of steroids.

To 1ml of extract glacial acetic acid, few drops of ferric chloride and then finally concentrated sulphuric acid were added from the walls of the test tube. Appearance of the reddish brown at the junction of two layers and the bluish green colour in the upper layer indicates the presence of cardiac glycosides.

5ml extract was boiled with 10ml of sulphuric acid and filtered while hot. The filterate was shaken with 5ml of chloroform the chloroform layer was pipetted out into another test tube then 1ml of dilute ammonia is added. The resulting solution was observed for colour changes. The change in colour indicates the presence of anthraquinones.

To one ml of the extract, a few drops of dilute sodium hydroxide are added. An intense yellow colour was produced in the plant extract, which became colorless on addition of few drops of dilute acid. This indicates the presence of flavonoids.

1ml of the extract was dissolved in 1ml of chloroform; 1ml of acetic anhydride was added following the addition of 2ml of concentrated sulphuric acid. Formation of reddish colour indicates the presence of terpenoids.

1ml of the extract was treated with few drops of Ninhydrin reagent. Appearance of purple colour indicates the presence of amino acids.

1ml of extract was added 5 to 10 drops of Fehling's solution. Mixture was then subjected to boiling for 15 minutes. Appearance of brick red precipitate indicates the presence of reducing sugars.

To the 1ml of extract, 1ml of Barfoed´s reagent was added and heated on water bath. Formation of brown precipitate indicates the presence of monosaccharides.

**Test organisms**

Microbial cultures obtained from the Department of Microbiology, University College of science, Osmania University, Hyderabad, India. Among seven bacterial species investigated, four gram negative bacteria (*E.coli*, *Psuedomonas*, *Salmonella typhi*, *Klebsiella pneumonia*), four gram positive bacteria (*Sterpococci*, *Bacillus subtilis*, *Bacillus cereus*) were carefully identified using standard microbiological methods. All the bacterial and fungal species were maintained at 4°C Nutrient agar.

**Preparation of concentrations**

Methanolic, ethanolic, acetone extracts of bark and leaf of *syzigium cumini* L were prepared as different concentrations (100µg/ml, 300mµ/ml, and 500µg/ml) to get the final drug concentration of 15µg/well,25µmg/well, and 75µg/well respectively, DMSO and standard streptomycin (10µg/ml) for bacteria we used as control. Concentrations of extracts were prepared by filter paper disc method; discs with 9mm diameter were prepared using No1 whatman filter paper and sterilized by autoclaving. Then, the discs had been impregnated with different concentrations of extracts.

**Determination of Minimum Inhibitory Concentration (MIC)**

Minimum Inhibitory Concentration was determined by micro dilution method using serial dilutions (2-fold) of plant extracts according to the National Committee for Clinical Laboratory standards (NCCL, 2000).The extracts were diluted to get series of concentration from100mg/ml to 12.5mg/ml in sterile nutrient broth. One drop of suspension of microorganisms was added to the broth dilutions. These were incubated for 18hours at $37^0$C.MIC of each extract was taken as the lowest concentration that did not give any visible bacterial growth. All experiments were performed in triplicate.

**Antibacterial activity**

Twenty four hours old cultures of the organisms were used to test the antibacterial activity. The nutrient agar medium plates were prepared by pouring 15ml of nutrient agar media into sterile Petri plates. The plates were allowed to solidify for 5 minutes and 0.1% inoculum was inoculated onto the solidified plates and allowed to dry for 5 minutes. Various concentrations of the plant extracts were transferred into the sterile filter paper discs and the plates were allowed to stand for one hour for pre-diffusion of extracts to occur. The plates were then incubated at $37^0$C for 24hours.At the end of incubation the inhibition zone developed measured. Triplicates were maintained and the averages of the zones of inhibition were calculated.

**Preliminary Phytochemical Tests**

A) -Yellow precipitate formed confirms the tannins

B) - Bluish green phenols confirms test

C) - Yellow colour steroids confirm test

D) - Reddish colour terpenoids confirmation test

E) - Brick red colour confirmation for carbohydrates

F) - Colour less solution Flavonoids confirmation test

G) -Formation of froth confirmation for saponins

H) -Bluish red Cardiac glycosides confirm colour test

I) - Bluish green confirmation for steroids

J) - Reddish yellow confirmation for anthraquinones

**Table 1: Phytochemical screening test of leaf extracts of *Syzygium cumini.***

| S. No | Phytochemicals | Methonol | Ethonol | Chloroform | Pet. ether | Water |
|---|---|---|---|---|---|---|
| 1. | Tannins | +++ | +++ | - | - | +++ |
| 2. | Phenols | +++ | +++ | - | - | ++ |
| 3. | Saponins | ++ | ++ | - | - | + |
| 4. | Alkaloids | ++ | + | - | - | - |
| 5. | Flavonoids | +++ | ++ | + | + | + |
| 6. | Anthraquinones | ++ | ++ | + | + | ++ |
| 7. | Aminoacids | - | - | - | - | - |
| 8. | Carbohydrates | +++ | +++ | - | - | ++ |
| 9. | Terpenoids | - | - | - | - | - |
| 10. | Cardiacglycosides | - | - | - | - | - |
| 11. | Steroids | ++ | ++ | + | + | + |

(+) = strongly present,(-) = absent

**Table 2: Antimicrobial Activity of Leaf Extracts of *Syzygium Cumini.***

| Solvents | Con. (µl) | Zone of inhibition in mm | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | E. coli | K. pneumonia | Streptococci | S. typhi | B. cereus | P. aeruginosa | Staphylococci | B. subtilis |
| Methanol | 5 | 14 | 3 | 7 | 15 | 9.6 | 6.3 | 14.3 | 9.8 |
| | 10 | 16 | 3 | 8 | 18 | 13 | 10.5 | 16 | 15.3 |
| | 15 | 19 | 8 | 11 | 20 | 15.1 | 16.8 | 17.5 | 18.1 |
| | Control* | 20.6 | 8 | 13.2 | 22 | 17 | 20 | 18.7 | 19.2 |
| Ethanol | 5 | 4.5 | 2 | 3 | 8.7 | 7.5 | 5.9 | 5 | 8.9 |
| | 10 | 6 | 3 | 5 | 10 | 10 | 9.6 | 6 | 13.8 |
| | 15 | 8 | 11 | 8 | 18.5 | 15 | 7.9 | 8 | 17 |
| | Control* | 20.6 | 8 | 13.2 | 22 | 17 | 15 | 18.7 | 19.2 |
| Pet ether | 5 | 3 | - | 2.5 | 2.4 | 2.8 | 3 | 1 | 4.4 |
| | 10 | 4.1 | - | 4 | 3 | 5 | 3.4 | 2.3 | 8.7 |
| | 15 | 5 | - | 6.5 | 4.5 | 6.3 | 4.7 | 3 | 9.5 |
| | Control* | 20.6 | 8 | 13.2 | 22 | 17 | 15 | 18.7 | 19.2 |
| chloroform | 5 | 1.5 | - | 1.3 | 0.5 | 3 | 1.6 | 0.5 | 2.9 |
| | 10 | 2.5 | - | 2 | 3 | 4 | 2 | 2 | 4.9 |
| | 15 | 3 | - | 3.5 | 3.8 | 4.6 | 3 | 2.5 | 5.0 |
| | Control* | 20.6 | 8 | 13.2 | 22 | 17 | 15 | 18.7 | 19.2 |

Control: streptomycin, concentration: µg/ml

## RESULTS

Table 1 shows the phytochemicals that are present in the leaf screened by different screening tests revealed the presence of tannins, phenols, saponins, carbohydrates commonly from methanol, ethanol and water that were not observed in chloroform and pet ether. Only the flavonoids, anthraquinones and steroids are commonly found in methanol, ethanol, pet ether, chloroform and water.

Table 2 explains the Antibacterial activity of *Syzygium Cumini* L leaf extracts was assayed. The data revealed that significant reduction in growth of bacteria was observed with leaf extracts of methanol, ethanol, pet ether and chloroform. And all the extracts showed significant differences in their efficacy. Among all the extracts methanolic extracts of wood were more prominent activity than the ethanol, pet ether, chloroform. Methanol, ethanol and pet ether extracts of leaf showed broad inhibition zone on all the bacteria tested than hot water and chloroform. The extracts of methanol and ethanol of leaf showed maximum activity even at lower concentrations. Methanol extracts of wood showed broad spectrum inhibition zone against the bacteria *Pseudomonas aeruginosa, E.coli, Salmonella typhi, Streptococci, Bacillus cereus* and *Bacillus subtilis.* Ethanol extracts showed maximum antibacterial activity in the order of *bacillus subtilis, bacillus cereus, klebsiella pneumonia, E.coli, streptococci and pseudomonas aeruginosa. Klebsiella pneumonia* is found to be inhibited by methanol and ethanol extracts only.

## DISSCUSSION

The result shows that most of the extracts showed the similar properties to the screening tests. It is recognized that terpenoids are attributed for analgesic and anti-inflammatory activities and flavonoids are reported to possess many useful properties, including antiinflammatory, oestrogenic, enzyme inhibition, antimicrobial, antiallergic, antioxidant, vascular and cytotoxic anti tumour activity. Anthocyanins can help the human immune system to protect against viral infections. Glycosides, flavonoids and alkaloids have shown hypoglycemic activities. Tannins inhibit the growth of different fungi, yeasts, bacteria and viruses (Chong *et al*.,2009). Saponins have been beneficial health effects and widely used as detergents, pesticides and industries as foaming and surface active agents (J. Shi, K. Arunasalam *et al* 2004). Phenols are have shown great importance as cellular support material because they form the integral part of cell wall structure by polymeric phenolics( Gupta, *et al* 2010) and they can protect humans from oxidative stress which may cause many

disease, including cancer, cardiovascular problems and ageing ( Robards and Prernzler *et al*, 1999) Due to the presence of phytochemicals in the wood of the *Syzygium Cumini* L have shown antimicrobial, antifungal, antidiabetic and anticancer activities. The present studies have justified the wood of the *Syzygium Cumini* L possess antimicrobial, antifungal activities based upon their dosage.

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