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PHYTOCHEMICAL AND ANTIBACTERIAL PROPERTIES OF ETHANOL EXTRACT OF *SYZYGIUM CUMINI* SEED ON PATHOGENIC BACTERIA

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ABSTRACT

Phytochemical investigation was carried out on the crude ethanol and aqueous extracts of *Syzygium cumini* . The antimicrobial activity of seed extract was tested against standard strains and clinical isolates of five bacteria using the agar well diffusion method. In Preliminary phytochemical analysis the presence of flavonoids, alkaloids, glycosides, steroids, phenols, saponins, terpenoid, cardiac glycosides and tannins were present in the extracts. The extracts showed inhibitory activity against pathogenic, gram negative bacteria viz, *Pseudomonas aeruginosa and Escherichia coli, Proteus vulgaris* and gram positive bacteria were *Bacillus subtilis, and Staphylococcus aureus.* The results showed that the ethanol extracts was more potent than the aqueous extracts.

The pharmacognosy implies a particular knowledge of methods of identification and evaluation of drugs. *Syzygium cumini* synonyms such as *Syzygium cumini* (L.) *Druce*, *Eugenia jambolana* Lam., *Syzygium jambolanum* DC, belongs to family Myrtaceae, is a large evergreen tree up to 30 m in height and a girth of 3.6 m with a bole upto 15 m found throughout India upto an altitude of 1,800 m (Chitnis *et al.*, 2012). It has been valued in Ayurveda and Unani system of medication for possessing variety of therapeutic properties.

The medicinal properties of several herbal plants have been documented in ancient Indian literature and preparations have been found to effective in treatment of disease. Therefore to meet the increasing demand of manufacturing modern medicine and export, the need of medicinal plants have enormously increases. This demand is generally met with by cultivating uprooted medicinal plants (Ahmed *et al.*,1998).

Staphylococcus infection can spread through contact with pus from infected wound, skin to skin contact with an infected person by producing hyaluronidase that destroyed tissue. *Proteus vulgaris* can be deadly when in sinus respiratory system, if left untreated or it is treated with antibiotic that have only intermediate effect *Proteus vulgaris* (Paratrick *et al.*, 1998). *Escherichia* genus which are found worldwide in warm band cold blooded animals, in humans, and in non living habitats. They cause illness in humans and many animals. *Pseudomonas aeruginosa* causes the urinary tract infections in human and animals, if remain untreated causes several complications related to UTI. Whereas *Bacillus subtillis* can contaminate the food however, they seldom results in food poisoning (Ryan and Ray, 2004).

During the last ten years pace of development of new antimicrobial drug has slows and the prevalence of resistance has increased surprisingly (Akinpelu and Onakoya, 2006). The problem of microbial resistance is increasing tremendously and antimicrobial drug becomes inactive against such resistant strain therefore action must be taken to reduce this problem, such as controlling the use of antibiotic and carrying out research of better understanding of genetic mechanism of resistance.

Researchers are increasingly turning their attention to herbal products such as *Syzigium cumini*, looking for new leads to develop better drugs against MDR microbe strains (Mohammed Imran *et al.*, 2017). In the present study; we have selected Indian medicinal plant *Syzigium cumini* to be screened against pathogenic bacteria. The selection of medicinal plants is based on its traditional uses in India. (Mehmoud *et al.*, 2001). The objective of this study was to determine the antibacterial effect of seed extracts from the *Syzigium cumini* seeds against pathogenic bacteria, *Escherichia coli* , *Staphylococcus aureus, Proteus vulgaris, Pseudomonas aeruginosa, Bacillus subtillis.*

MATERIAL AND METHODS

The experiment was conducted in Microbiology Laboratory, Department of Microbiology, Shri Radhakisan Laxminarayan Toshniwal College of

Science, Akola, for six months (October 2017-March 2018).

The seed of *Syzygium cumini* were collected from local market of Akola and were shade dried, powdered and extracted in soxhlet apparatus successively with ethanol and aqueous respectively due to their nature of polarity. After extraction, the ethanol and aqueous extracts were filtered through Whatman No.1 filter paper and stored for further use.

The seed extracts of *Syzygium cumini* were analyzed for the presence of flavonoids, alkaloids, glycosides, steroids, phenols, saponins, terpenoid, cardiac glycosides and tannins according to standard methods (Harborne, 1973).

Mercuric chloride 1.36 gm dissolved in 60ml and 5gm of potassium iodide dissolved in 10 ml of distilled water. These two solvents were mixed and diluted to 100ml using distilled water. To 1ml of acidic aqueous solution of samples few drops of above reagent was added. Formation of white or pale precipitate showed the presence of alkaloids.

In a test tube containing 0.5ml of alcoholic extract of the samples, 5 to 10 drops of diluted HCl and small amount of Zn or Mg were added and the solution was boiled for few minutes. Appearance of reddish pink or dirty brown colour indicated the presence of flavonoids.

Small amount of alcoholic extract of samples was dissolved in 1ml water and then aqueous sodium hydroxide was added. Formation of a yellow colour indicated the presence of glycosides.

About 100mg of dried extract was dissolved in 2ml of chloroform. Conc. Sulphuric acid was carefully added to form a lower layer. A reddish brown colour at the interface was an indicative of the presence of steroidal ring.

Seed extract 100 mg was dissolved in 10ml of glacial acetic acid containing one drop of ferric chloride solution and 1ml of concentrated sulphuric acid was added. A brown ring obtained at the interface indicated the presence of a de oxy sugar characteristic of Cardiac glycosides.

A drop of sodium bicarbonate was added in a test tube containing about 50ml of an aqueous extract of sample. The mixture was shaken vigorously and kept for 3min. A honey comb like froth was formed and it showed the presence of saponins.

Chloroform or ethanol extract of 2ml and addition of 5 to 10ml of acetic anhydrite was dissolved by gentle heating. After cooling, 0.5ml of H_2SO_4 was added. Bright purple colour was produced. It indicated the presence of resins.

Alcoholic solutions 1ml of sample, 2ml of distilled water followed by a few drops of 10% aqueous ferric chloride solution was added. Formation of blue or green colour indicated the presence of phenols.

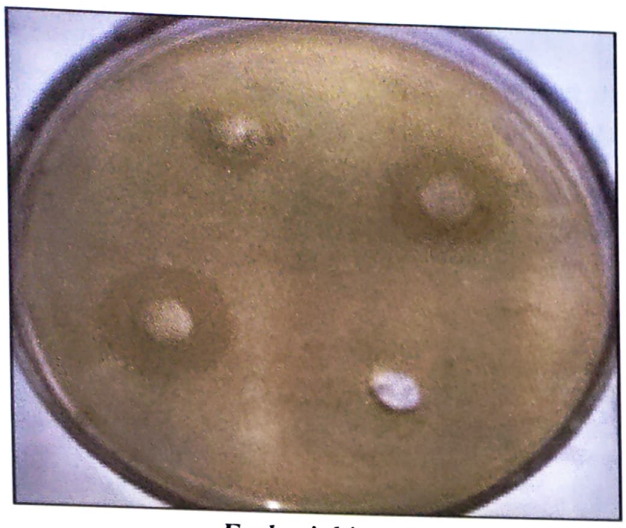
In a test tube containing about 5ml of an aqueous extract, a few drops of 1% solution of lead acetate was added. Formation of a yellow or red precipitate indicated the presence of tannins. A 2ml filtrate [200mg of plant material in 10ml distilled water, filtered], and 2ml of $FeCl_3$ were mixed. A blue or black precipitate indicates the presence of Tannins. And 2ml of chloroform and 1ml of conc. H_2SO_4 was added to 1mg of extract and observed for reddish brown colour that indicated the presence of terpenoid.

Gram negative bacteria viz. *Pseudomonas aeruginosa, Escherichia coli* and *Proteus vulgaris*. Gram positive bacteria as *Staphylococcus aureus, Bacillus subtilis,* were procured from Department of Microbiology of Shri R. L. T. College of Science, Akola, Maharashtra.

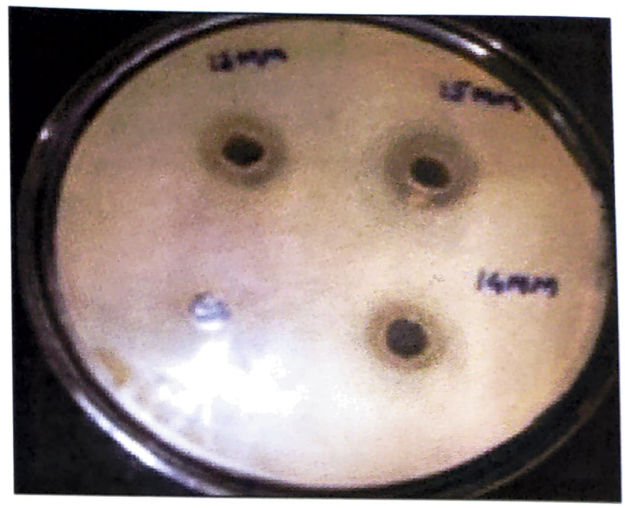
Muller Hinton media is selectively used for antimicrobial activity and antibiotic sensitivity assays. It allows growth of microorganisms. It shows better diffusion of extract which gives true zone of inhibition by microorganisms against extracts. It contains starch which absorbs toxic metabolites produced by bacteria so that they cannot interfere with the activity of extract. The petriplates and the Muller Hinton medium were sterilized. The rest of the procedure was carried out in laminar air flow. Approximately 20ml of the media was poured into the sterile petriplates and allowed to get solidified. After the media gets solidified the bacterial organisms were inoculated.

Antibacterial Assay

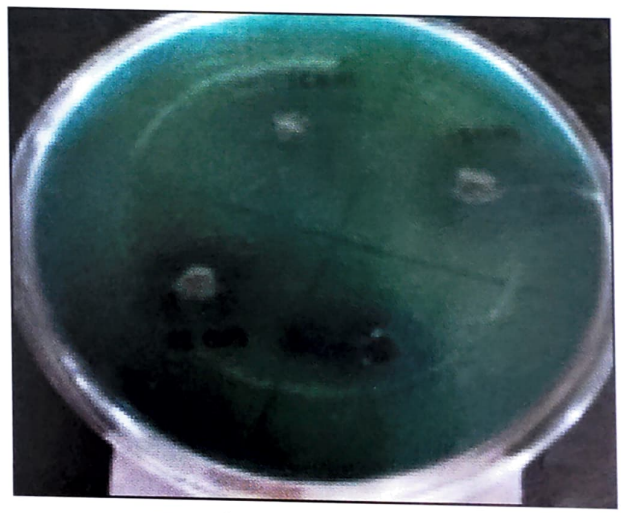
The agar well diffusion method (Tahir *et al.,2012*) as adopted earlier (Ahmad *et al.*, 1998) was used. 0.1 ml of diluted inoculums (10^5 CFU/ml) of test organism was spread on Muller- Hinton agar plates. Wells of 6 mm diameter was punched into the agar medium and filled with 25 il, 50 il, 75 il, 100 il of *Syzigium cumini* seed extract of 100 mg/ ml concentration. The plates were incubated for overnight at 37°C. The antibacterial activity was evaluated by measuring the zone of inhibition against



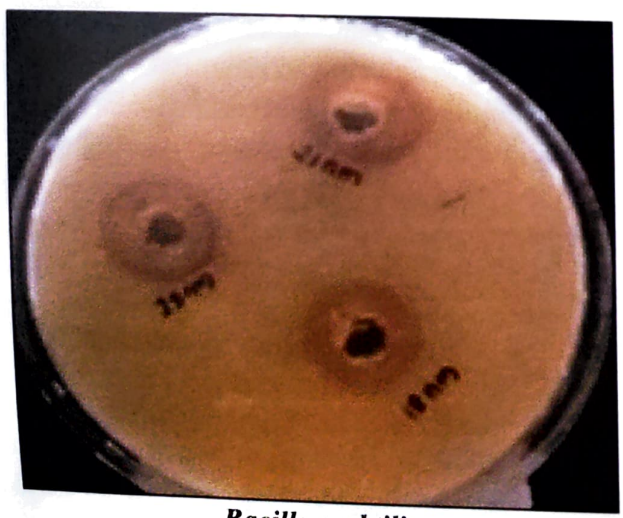
Escherichia coli



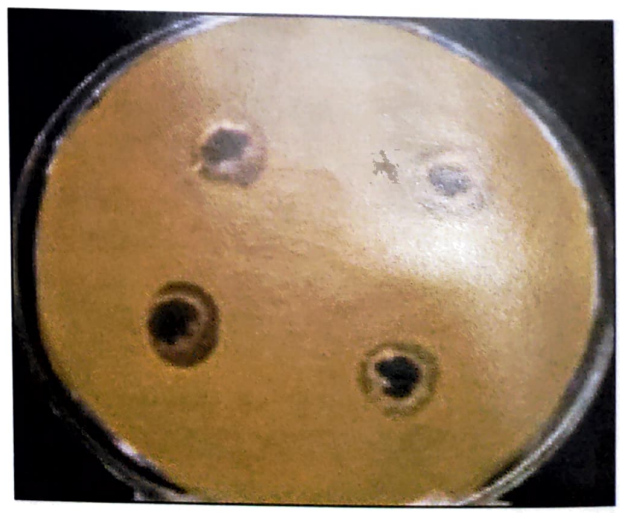
Staphylococcus aureus



Pseudomonas aeruginosa



Bacillus subtilis



Proteus vulgaris

Plate 1 : Antimicrobial activity of seed extracts at different concentration against pathogenic bacteria

test organism. The antibiotic disc to which chloramphenicol (30ig) was used in the test system as positive controls. Each experiment was performed in triplicate.

RESULTS AND DISCUSSION

The phytochemical screening and antibacterial activities were performed with methanol and aqueous extracts of the seed of *S.cumini*. The study was made against two gram positive pathogenic bacteria and three gram negative bacteria using the standard agar well diffusion method. The seed of *S. cumini* were rich in flavonoids, alkaloids, glycosides, steroids, phenols, tannins and saponins. These phytochemicals confer antimicrobial activity on the seed extracts (Table-1).

Table 1. Phytochemical screenings of *Syzygium cumini* seed extracts.

Phytochemical constituents	Ethanol	Aqueous
Flavoniods	+++	+++
Alkaloids	++	+++
Glycosides	++	++
Steroids	+++	+
Phenols	++	+++
Terpenoids	+	++
Saponins	+	+
Resins	+	+
Tannins ($FeCl_3$ test)	+	+
Tannins (Lead acetate test)	+	+
Cardiac glycosides	+	++

+ = present, ++ = moderately present,
+++ =Appreciable amount

The various phytochemical compounds detected are known to have beneficial importance in medicinal sciences. For instance, flavonoids have been referred to as nature's biological response modifiers, because of their inherent ability to modify the body's reaction to allergies and virus and they showed their anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activities (Aiyelaagbe and Osamudiamen 2009). Plant steroids are known to be important for their cardio tonic activities and also possess insecticidal and antimicrobial properties. They are also used in nutrition, herbal medicine and cosmetics (Gowari and Vasantha 2010).

In ethanol extract presence of flavoniods and steroids was appreciably indicated in ethanol while in aqueous extract in addition to these appreciable presence of alkaloids was observed. The presence of alkaloids, glycosides and phenols were moderately present in ethanol extract whereas glycosides, terpenoids, cardiac glycosides were obsereved to be present moderately in aquous exrtract .Where as presence of saponins, resins and tannins mere of equal level in both aqueous and ethanol extract. Tannins were reported to exhibit antiviral, antibacterial and anti-tumor activities. It was also reported that certain tannins were able to inhibit HIV replication selectively and was also used as diuretic (Yadav *et al.*, 2011). Saponin is used as mild detergents and in intracellular histochemistal staining. It is also used to allow antibody access in intracellular proteins. In medicine, it is used in hypercholesterolemia, hyperglycemia, antioxidant, anticancer, anti-inflammatory, weight loss, etc. It is also known to have antifungal properties (Halsem *et al.*, 1989).

The results of the ethanol and aqueous extract of seed exhibited antibacterial activity against all the tested strain viz. *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli and Proteus vulgaris* (Table 2).

The zones of inhibitions were produced by both the aqueous and ethanol extracts against all the test organisms, plate 1. The zones of inhibition were ranging from 6-19mm in diameter. Ethanol extracts was more active than the aqueous extract against *B.subtilis* at 50, 75,100 il and have shown maximum inhibition activity of 12-19mm zone and it was outstanding against the remaining test bacteria. Likewise the antibacterial activity of ethanol extract was also better against *E.coli* has it caused max. Inhibition from 13-16 mm. For inhibition of *P.aeruginosa* and *P.vulgaris* it was more or less equal ranging from 13-17 mm for 75-100 il. However. It was prominently observed for antibacterial activity of ethanol and aquous extract w Equal antibacterial activity of seed extract at lowest quantity of 25-75 il shows inhibition up to 8-14mm in both in ethanol and aqueous and ethanol extract for *S. aureus*. However for 100 il inhibition was more in aqueous than ethanol extract. The highest zone of inhibitions (19 mm) noted in ethanol extract against *Bacillus subtilis* with 100il concentration. The extracts of higher plants can be very good source of antibiotics (Yadav *et al., 2011*) against various bacterial pathogens.

Plant having antimicrobial compounds have enormous therapeutic potential as they can act without any side effect as often found with synthetic antimicrobial products. Most antimicrobial medicinal plant are more effective against gram-positive than gram-negative bacteria (Lin *et al.*, 1999). However current findings

Table 2. Antibacterial activity of *Syzygium cumini* seeds using agar well diffusion method

Extracts	Indicator Bacteria	Antibacterial activity of seed extract (il)* zone of inhibition in mm				
		Control	25	50	75	100
Ethanol Extract	*Staphylococcus aureus*	5.00	8.00	10.00	14.00	16.00
	Bacillus subtilis	5.00	7.00	12.00	15.00	19.00
	Escherichia coli	5.00	5.00	9.00	13.00	16.00
	Pseudomonas aeruginosa	5.00	6.00	8.00	13.00	17.00
	Proteus vulgaris	5.00	4.00	8.00	15.00	17.00
Aqueous Extract	*Staphylococcus aureus*	8.00	8.00	10.00	14.00	18.00
	Bacillus subtilis	5.00	7.00	11.00	12.00	15.00
	Escherichia coli	5.00	5.00	8.00	12.00	14.00
	Pseudomonas aeruginosa	5.00	8.00	12.00	14.00	17.00
	Proteus vulgaris	5.00	6.00	10.00	15.00	17.00

Control: Chloramphenicol (30Ig)

showed a remarkable activity against gram-negative bacteria. The antimicrobial activity of the *S.cumini* seeds ethanol and aqueous extract may be due to tannins and other phenolic constituents. *S.cumini* is known to be very rich in gallic and ellagic acid polyphenol derivatives (Chattopadhyay *et al.*,1998 and Chung *et al.*,1998). Acylated flavonol glycosides, kaempferol, myricetin and other polyphenols were isolated from *S.cumini* leaves (Timbola *et al.*, 2002).Tannins are considered nutritionally undesirable because they precipitate proteins, inhibit digestive enzyme and affect the absorption of vitamins and minerals. However, some kinds of tannins can reduce the mutagenicity of a number of mutagens and display anticarcinogenic, antimicrobial and antioxidant activities (Chung *et al.*,1998).

The result obtained in this study suggests a potential application of *S. cumini seeds* for treatment of skin infection; UTI needs further investigations in order to explore their applications. Other medicinal plants containing phenolic compounds, including tannins, as major constituents are used topically for care and repair of skin wounds.The advantage of the use of topical antimicrobials is their ability to deliver high local concentrations of antibiotic irrespective of vascular supply. Further benefits include the absence of adverse systemic effects, and a low incidence of resistance (Spann *et al.*,2003).

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