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ANTIBACTERIAL ACTIVITY FROM LEAF EXTRACT OF *Syzygium Cumini L.*

KANSAGRA K[1], JAGAD M[2] AND RAM D[3*]

1, 2: Under Graduate Student, Noble Pharmacy College-Junagadh, Gujarat Technological University-Ahmedabad

3: Associate Professor, Faculty of Pharmacy, Noble University, Junagadh

[*]**Corresponding Author: Dr. Darshit Ram: E Mail: Jmeet130@gmail.com**

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Abstract

Purpose: This study is carried out for the purpose to investigate the antibacterial activity of *Syzygium cumini L*. Leaves. The aim of the study is to evaluate the antibacterial activity and to determine the zone of inhibition of alcoholic extract on some bacterial strains.

Methodology: In the present study, microbial activity against the medically important bacteria is determined using the methanolic extract of *Syzygium cumini L*. leaves by using agar disc diffusion method.

Findings: It shows antibacterial activity against 2 Gram positive bacteria - Staphylococcus aureus, Bacillus cereus and 2 Gram negative bacteria Alcaligenes faecalis, Aeromonas hydrophilia pathogenic bacteria. Zone of inhibition of alcoholic extract of leaves is compared with the standard drugs like chloramphenicol, ampicillin, cotrimoxazole and vancomycin for antibacterial activity. After practical, it is observed that the remarkable activity against microbes is shown by the alcoholic extract of the leaves of *Syzygium cumini L*. The phytochemical of plant is analyzed. The antibacterial activity of leaves due to secondary metabolites present in the *Syzygium cumini L.* Plant. So, this plant could be useful to discover new antibacterial natural

pharmaceutical compound.

Unique contribution to theory, practice and policy: So, this plant could be useful to discover new antibacterial natural pharmaceutical compound.

Keywords: *Syzygium cumini L.*, zone of inhibition, antibacterial activity, Leaf extract, Gram-positive bacteria, Gram-negative bacteria

1. INTRODUCTION

Antibiotics are one of the most important weapons for fighting bacterial infections and have greatly benefited the human health since they are discovered. But, over the past few decades, these benefits are under threat as many commonly used antibiotics have become less effective against certain illnesses. This is because many of them produce toxic reactions and emergence of drug-resistant bacteria. It is essential to find newer drugs with lesser resistance. Drugs derived from natural sources play a crucial role in the prevention and treatment of human diseases. In many developing countries, traditional medicine is the primary healthcare systems **[1, 2]**. Recent trends show that the discovery rate of active novel chemical entities has been declined **[3]**. Natural products of plants may give a new source of antimicrobial agents with possibly novel mechanisms of action **[4, 5]**. The effects of plant extracts on bacteria have been studied by many researchers in different parts of the world **[6]**. Plants are rich in secondary metabolites such as tannins, terpenoid alkaloids, flavonoids, glycosides, steroids, cardiac glycoside, saponins, resins, phenols, etc., which have been found in vitro antimicrobial properties **[7]**. Herbal medicines have been known to us for centuries. Therapeutic efficacy of many indigenous plants for several disorders has been described by practitioners of traditional medicine **[8]**.

Antimicrobial properties of medicinal plants have been highly reported from different parts of the world. The World Health Organization (WHO) estimates that plant extracts or their active constituents are used in traditional therapies of 80% of the world's population **[9]**. The harmful microorganisms can be controlled with drugs and this creates a need for the emergence of multiple drug-resistant bacteria and it has created alarming clinical situations for the treatment of infections. The

pharmacological industries have produced many new antibiotics; resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to synthetic drugs which are used as therapeutic agents **[10]**. To expand the spectrum of antibacterial agents from natural resources, *Syzygium cumini L*. belonging to *Mytraceae* family has been selected. In the Indian literature, this plant has been described to be useful against skin diseases, liver issues, tuberculosis glands and its use into the treatment of hematemesis, pruritus, Leukoderma, and diabetes has been suggested **[11, 12]**.

Besides this, the plant extract is also recommended as a pest and disease control agents in India **[13-15]**. The leaves are antihyperglycemic, hypolipemiant, anti-inflammatory, cardioprotective, and antioxidant activities and are used in dermopathies, gastropathies, constipation, leucorrhea, and daibetes. *Syzygium cumini L*. plant organs are known to be an important source of secondary metabolites, notably phenolic compounds. Indian people are using the leaves to treat inflammation; *Syzygium cumini L* **[16]**. *Syzygium cumini L*. exhibited significant antimicrobial activity and showed properties that support folkloric use in the treatment of some diseases as broad-spectrum antimicrobial agents **[17]**. Thus, *Syzygium cumini L*. is well anchored in its traditional uses and has now found wide-spread acceptance across the world. In the current investigation carried out, a screening of hydroalcoholic extracts of *Syzygium cumini L*. leaves against pathogenic bacteria is done in order to detect new sources of antimicrobial agents.

2. MATERIALS AND METHODS

2.1 Collection of Plant Materials

The leaves of *Syzygium cumini L* were collected in October 2008 from semi-arid region of Junagadh, Gujarat, India. The leaves were separated, washed thoroughly with tap water, shade dried, homogenized to fine powder, and stored in an airtight bottle and the bottle are numbered, noted with the date of collection, locality, and their medicinal uses were recorded **[18]**.

2.2 Preparation of leaves Extract Extraction

2.2.1 Decoction Extraction Method

For the decoction method, 5g of dried leaf powder was extracted with 100 ml of deionized water for 30 min at 100º C in a water bath. Then the extract was filtered with eight layers of muslin cloth and centrifuged at 5,000 rpm for 10 min. The supernatant was collected and the solvent was evaporated using a rotary vacuum evaporator (Equitron, India). The extract was stored at 4 °C in an airtight bottle. The residue was weighed and the extractive yield was obtained.

2.2.2 Maceration Extraction Method

For maceration method, 5 g of dried leaf powder was extracted with 100 ml of 50% aqueous ethanol at 25 °C for 42 h in static condition. The extract was filtered with eight layers of muslin cloth and centrifuged at 5,000 rpm for 10 min. The supernatant was collected, and the solvent was evaporated using a rotary vacuum evaporator (Equitron, India). The extract was stored at 4 °C in an airtight bottle. The residue was weighed and the extractive yield was obtained. Five grams of dried leaf powder was extracted with 100 ml of 80% aqueous methanol at 35 °C for 24 h in an incubator (Cai *et al*. 2004). The extract was filtered with eight layers of muslin cloth and centrifuged at 5,000 rpm for 10 min. The supernatant was collected, and the solvent was evaporated using a rotary vacuum evaporator (Equitron, India) to dryness. The extract was stored at 4 °C in an airtight bottle. The residue was weighed and the extracted yield was obtained **[18]**.

2.3 Preliminary Phytochemical Screening The extracts were subjected to preliminary phytochemical testing for the presence of different chemical groups of compounds. Air-dried and powdered plant materials were screened to detect the presence of saponins, tannins, alkaloids, flavonoids, terpenoids, steroids & glycosides as described in literatures **[7]**.

2.4 Test Microorganisms and Growth Media

2.4.1 The following microorganisms *Aeromonas hydrophilia, Alcaligenes faecalis, Bacillus cereus, Citrobacter freundii, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas fluorescens, Salmonella newport, Serratia marcescens, Shigella sonnei, Staphylococcus aureus, Staphylococcus epidermidis* and *Streptococcus pyogenes* were chosen based on

their clinical and pharmacological importance **[19]**. The bacterial strains obtained from Institute of Microbial Technology, Chandigarh, were used for evaluating antimicrobial activity. The bacterial cultures were incubated for 24 hours at 37°C on nutrient agar and potato dextrose agar (PDA) medium (Microcare laboratory, Surat, India), respectively, following refrigeration storage at 4°C. The bacterial strains were grown in Mueller-Hinton agar (MHA) plates at 37°C (the bacteria were grown in the nutrient broth at 37°C and maintained on nutrient agar slants at 4°C), whereas the yeasts and molds were grown in Sabouraud dextrose agar and PDA media, respectively, at 28°C. The stock cultures were then maintained at 4°C **[20]**.

2.4.2 Preparation of standard bacterial suspensions

A suspension containing about (108 -109) colony forming unit per ml. was prepared. Dilutions of stock suspension were made in sterile normal saline (0.02ml) volumes were transferred into the surface of dry nutrient agar plates. The plates were allowed to stand at room temperature for 2 hours and the number of developed colonies per drop (0.02ml) was multiplied by 50 and by the dilution factor to give the viable count of the stock suspension known as the number of colony forming unit per ml (CFU/ml) of suspension. Each time a fresh stock suspension was prepared, all above experiment condition were maintained constant so that very accurate viable counts would be obtained **[21]**.

3. Antimicrobial Activity

3.1 Determination of zone of inhibition method

In vitro antibacterial activity was examined for hydroalcoholic extracts. Antibacterial activities of plant part extracts against four pathogenic bacteria (two Gram-positive and negative) which were investigated by the agar disk diffusion method **[22-24]**. Antimicrobial activity testing was carried out by using agar cup method. Each purified extracts were dissolved in dimethyl sulfoxide, sterilized by filtration using sintered glass filter, and stored at 4°C. For the determination of zone of inhibition, Gram-positive & Gram-negative strains were taken as a standard antibiotic for comparison of the results **[21]**.

All the extracts were screened for their antibacterial activities against the *Alcaligenes faecalis, Aeromonas hydrophilia, Staphylococcus aureus, Bacillus cereus*. The sets of five dilutions (5, 25, 50, 100, and 250 µg/ml) of *Syzygium cumini L*. extract and standard drugs were prepared in double distilled water by using nutrient agar tubes. Mueller-Hinton sterile agar plates were seeded with indicator bacterial strains and allowed to stay at 37° C for 3 hours. Control experiments were carried out under the same or similar conditions by using chloramphenicol, ampicillin, cotrimoxazole and vancomycin for antibacterial activity as standard drugs. The zones of growth inhibition were measured after 18 to 24 hours of in incubation at 37°C for bacteria. The sensitivities of the microorganism species to the plant extracts were determined by measuring the sizes of inhibitory zones (including the diameter of disk) on the agar surface and values <8 mm was considered as not active against microorganisms.

4. RESULTS AND DISCUSSION

4.1 Results

4.1.1 Preliminary phytochemical screening

It was found that hydroalcoholic extracts of *Syzygium cumini L*. leaves contained tannins, terpenoid alkaloids, flavonoids, glycosides, steroids, cardiac glycoside, saponins, resins, phenols, etc.

4.1.1 Microbial activity

The antimicrobial activity of the extracts of *Syzygium cumini L*. leaves were studied in different concentrations (5, 25, 50, 100, and 250 µg/ml) against four pathogenic bacterial strains, two Gram-positive (*Staphylococcus aureus, Bacillus cereus*) and two Gram-negative (*Alcaligenes faecalis, Aeromonas hydrophilia*). These strains have been selected based on application purpose of further formulation study.

Antibacterial potential of extracts was evaluated in terms of zone of inhibition of bacterial growth. The results of the antibacterial activities are presented in **Tables 1 and 2**.

The antibacterial and antifungal activities of the extracts increase with the increase in concentration of extracts (µg/ml). As compared with standard drugs, the results revealed that in the extracts for bacterial activity, *Alcaligenes faecalis* and *Aeromonas*

hydrophilia were more sensitive as compared with *S. aureus, B. cereus*. The growth inhibition zone measured was ranged from 11 to 20 mm for all the sensitive bacteria, **(Figures 1-4)**.

The results show that the extracts of *Syzygium cumini L*. leaves were found to be more effective against all the microbes tested.

Table 1: Antibacterial activities of hydroalcoholic extracts of leaves of *Syzygium cunmini L.* against bacterial test organism

Antibacterial activity (Zone of inhibition)					
Microorganisms	***Syzygium cumini* – zone of inhibition in mm**				
	Hydroalcoholic extract (concentration in µg/ml)				
	5	**25**	**50**	**100**	**250**
Alcaligene faecalis	**-**	**16**	**17**	**18**	**20**
Aeromonas hydrophilia	**-**	**14**	**15**	**16**	**19**
Bacillus cereus	**-**	**15**	**16**	**17**	**20**
Staphylococcus aureus	**-**	**13**	**14**	**15**	**18**

Table 2: Antibacterial activity of standard drugs against bacterial test organism

Antibacterial activity (Zone of inhibition)					
Drug	**Concentration (µg/ml)**	**Zone of inhibition in (mm)**			
		A. faecalis	***A. hydrophilia***	***B. cereus***	***S. aureus***
Ampicillin	**5**	**14**	**11**	**12**	**15**
	25	**15**	**12**	**13**	**16**
	50	**16**	**14**	**14**	**18**
	100	**18**	**15**	**16**	**19**
	250	**20**	**16**	**18**	**21**
Chloramphenicol	**5**	**15**	**17**	**14**	**16**
	25	**16**	**18**	**15**	**17**
	50	**17**	**19**	**17**	**18**
	100	**19**	**21**	**18**	**20**
	250	**20**	**23**	**20**	**23**
Cotrimoxazole	**5**	**18**	**17**	**16**	**15**
	25	**19**	**18**	**17**	**16**
	50	**21**	**19**	**18**	**18**
	100	**22**	**20**	**19**	**20**
	250	**24**	**22**	**20**	**21**
Vancomycin	**5**	**16**	**15**	**18**	**17**
	25	**17**	**16**	**19**	**18**
	50	**18**	**17**	**21**	**19**
	100	**20**	**18**	**23**	**21**
	250	**21**	**19**	**26**	**22**

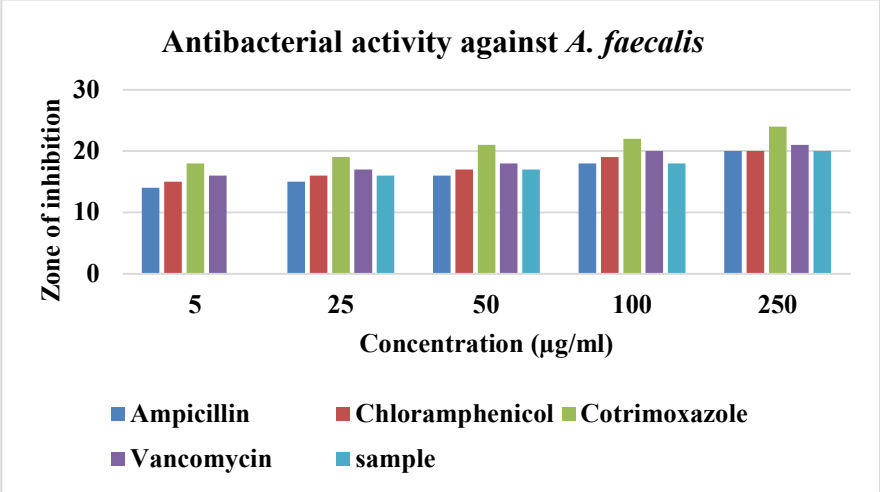

Figure 1: Antibacterial activity against *A. faecalis*

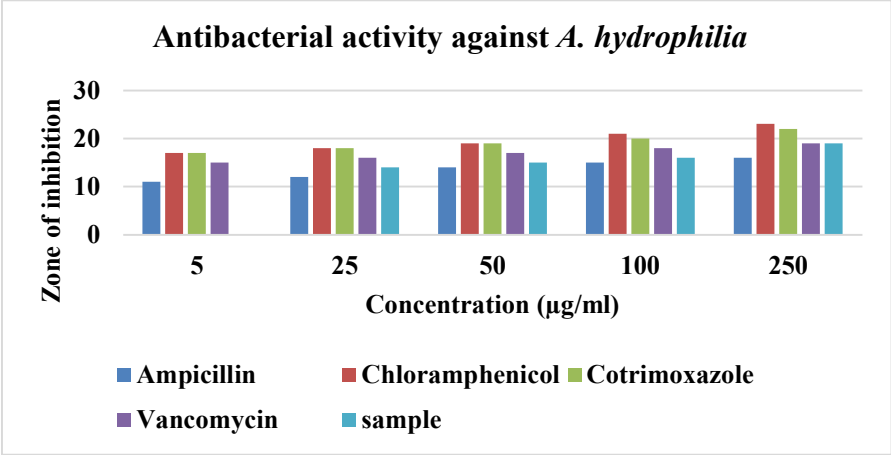

Figure 2: Antibacterial activity against A. hydrophilia

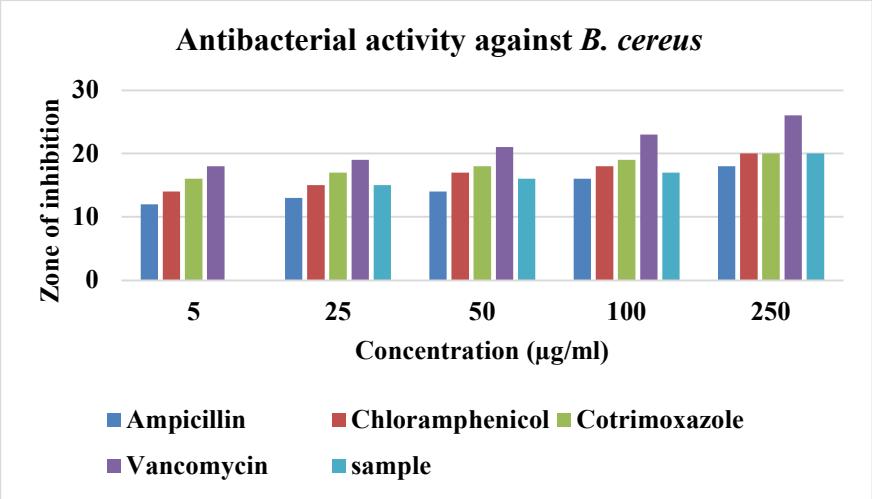

Figure 3: Antibacterial activity against *B. cereus*

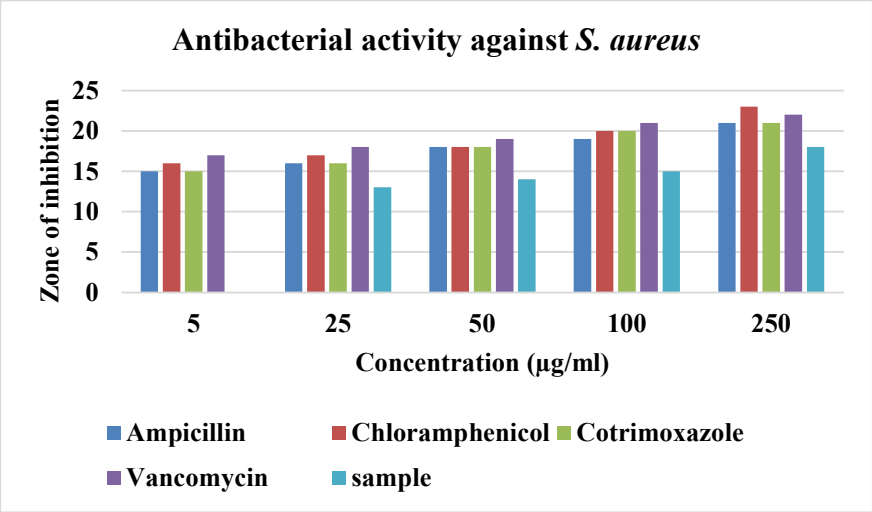

Figure 4: Antibacterial activity against *S. aureus*

4.2 DISCUSSION

Antimicrobial properties of medicinal plants are being hugely reported from different parts of the world. The World Health Organization estimates that plant extract or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population. In the present work, the extracts obtained from *Syzygium cumini L.* show strong activity against most of the tested bacterial strains. The results were compared with the standard antibiotic drugs. In this screening work, extracts of *Syzygium cumini L.* were found to be not inactive against any organism, such as Gram-positive, Gram-negative strains were resistant to all the extracts of *Syzygium cumini L.*

The results show that the activity of hydro alcoholic extracts of *Syzygium cumini L.* shows significant antibacterial activities. This study also detects the presence of different phytochemicals with biological activity that can have valuable therapeutic index. The result of phytochemicals in the present investigation showed that the plant contains more or less similar components like tannins, terpenoid alkaloids, flavonoids, glycosides, steroids, cardiac glycoside, saponins, resins, phenols, etc. Results show that plant rich in flavonoid, alkaloids and phenolic compounds may show antimicrobial activities against a number of microorganisms.

5. CONCLUSION

In the current investigation, the hydroalcoholic extract in the ratio of 8 : 2 has been selected after study of such a selected

plant with water extracts and methanol extracts, hydroalcoholic extract gave higher yield of chemical constituents expected for this research work; the originality of this work is that good results have been found with hydroalcoholic ratio, and it will be helpful to carry out other data with MIC and other formulation study, because in comparison of methanol or water extracts, hydroalcoholic is more suitable for clinical study. The hydroalcoholic extract of *Syzygium cumini L*. were found to be active on most of the clinically isolated bacteria, as compare with standard drugs. This study justified the claimed uses of leaves in the traditional medicine to treat various infectious disease caused by the microbes. However, further studies are needed for better evaluation of the potential effectiveness of the crude extracts as the antimicrobial agents. The present results will form the base for selection of plant species for further investigation in the potential discovery of new natural bioactive compounds. Further studies which aimed at the isolation and structure explanation of antibacterial active constituents from the plant have been initiated.

6. ACKNOWLEDGEMENT

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