

Antimicrobial activity of the various extracts from *S. cumini* (Bark)

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Abstract: *Syzygium cumini* is a well known traditional medicinal plant. The phytochemical Constituents of the plant are responsible for its medicinal properties. Investigations were carried out on the crude methanol, ethanol and aqueous extracts of the bark of *Syzygium cumini* (Magnoliopsida: Myrtaceae). The antimicrobial activity of the extract was tested against standard strains of bacteria using the agar well diffusion method. Phytochemical studies showed the presence of flavonoids, alkaloids, glycosides, steroids, phenols, saponins, terpenoid, cardiac glycosides and tannins as the class of chemicals present in the extracts of bark. The extracts reflected inhibitory activity against clinical isolates.

The gram negative bacteria such as *Salmonella typhi*, *Pseudomonas aeruginosa* and *Escherichia coli*; and the gram positive bacteria like *Bacillus subtilis* and *Staphylococcus aureus* were inhibited with the methanol and ethanol extract on contrary to aqueous extract. The methanolic extract of seed was found more potent antimicrobial agent than the leaves and bark extract.

The present study provides a support for the use of different part of *Syzygium cumini* as potent antimicrobial agents for sustainable and ecofriendly management of various bacterial strains and further investigation are required for field application.

Key Words: *Syzygium cumini* Bark extract, Phytochemical screening, Antibacterial activity.

1. INTRODUCTION:

An antimicrobial is a substance that kills or inhibits the growth of microorganisms such as fungi, bacteria as well as destroying viruses. Antimicrobial drugs kill microbes or prevent the growth of microbes. It is concerned with their form, structure, reproduction, physiology, metabolism and identification (Ramseo *et al.* 1946).

Microorganisms are available naturally in the surrounding environment; therefore they can easily reach food during harvesting, slaughtering, processing, and packaging (Hatab *et al.*, 2016). These microorganisms can survive under adverse conditions used in the food preservation such as low temperature, modified atmosphere packaging, vacuum packaging, as well as resist conventional pasteurization (Dimitrijević *et al.*, 2007; Provincial *et al.*, 2013; Saraiva *et al.*, 2016; Säde *et al.*, 2017). Thus, there is a considerable concern among consumers regarding the risk of using synthetic additives for human health, that led to decrease the use of these chemicals in food preservation (Gyawali and Ibrahim, 2014; Caleja *et al.*, 2016; Kalem *et al.*, 2017). Therefore, new eco-friendly methodologies are required to reduce the growth of pathogenic bacteria and prolong the shelf-life of food products, without using chemical preservatives. Recently, many researchers investigated the possible utilization of some plant extracts as effective natural preservatives (Fernández-López *et al.*, 2005; Suppakul *et al.*, 2016; Clarke *et al.*, 2017). Traditionally, the crude extracts of different parts of medical plants, including root, stem, flower, fruit, and twigs, were widely used for treatments of some human diseases (Khan *et al.*, 2013). Medicinal plants contain several phytochemicals such as flavonoids, alkaloids, tannins, and terpenoids, which possess antimicrobial and antioxidant properties (Talib and Mahasneh, 2010).

The antimicrobial activities of some plant species have been widely researched. For example, the crude extracts of cinnamon, garlic, basil, curry, ginger, sage, mustard, and other herbs exhibit antimicrobial properties against a wide range of Gram-positive and Gram-negative bacteria (Alzoreky and Nakahara, 2003; Castro *et al.*, 2008). In addition, it has been reported that the extracts from Chinese chives and cassia can effectively reduce the growth of *Escherichia coli* and other bacteria during storage of meat, juices, and milk (Mau *et al.*, 2001). In a similar study, Doddanna *et al.*

(2013) investigated the effect of some plant extracts on the growth of *Candida albicans*; the results indicated that the alcoholic extract of curry leaves effectively inhibit the growth of *C. albicans* with 24.05 ± 0.07 after 48 h. Moreover, Nzeako et al. (2006) reported that thyme oil extract could decrease the growth of *C. albicans* and *Pseudomonas aeruginosa*.

Plants have been used as a source of medicines for chronic and infectious disorders since time immemorial. In present time, herbal medicines are so important because of the side effects of synthetic pharmaceutical products and the safety, efficiency and promising potential of plant derived medicine. Researchers are focused on screening methods used for identification of potential antimicrobial plants. In this study diffusion methods as qualitative technique give an idea of the presence or absence of substances of antimicrobial activity in plant extracts.

On the other hand, dilution methods are considered quantitative assays once they determine the minimal inhibitory concentration. Tumors as well as age dependent diseases such as atherosclerosis, arthritis, and neuro-degenerative disorders associated with generation of oxygen free radical, reactive oxygen species (ROS), and hydrogen peroxide. The chemo-preventive role present in consumable fruits, vegetables and beverages so it is essential to discover and characterize antioxidants from natural origin. Three following plant extracts has been used for the assessment of antioxidant potential based on DPPH free radical scavenging method (Lee et al., 1998). *Zanthoxylum armatum* DC. (Fam. Rutaceae), known as tajbal in Hindi. This herb used as hepatic tonic, anthelmintics, antiviral and mosquito repellent etc. (Gogte, 2000; Kokate, 2001; Kumar & Müller, 1999). *Swertia chirata* Buch. & Ham. (Gentianaceae) has important medicinal and pharmaceutical values. The root of Chirata possesses significant antipyretic, analgesic properties and a high rise therapeutic clue (Tabassum et al., 2012). *Terminalia bellerica* Roxb. (Combretaceae) seed oil is used to cure skin diseases, premature graying of hair and can be applied on painful swollen parts. The fruits of *T. bellerica* can be used to treat cough, cold, hoarseness of voice, asthma, arrest bleeding, boost hair growth, impart black colour to hair, cure conjunctivitis, astringent and anti-diarrheal agent. It also helps in loss of appetite, piles, lowering cholesterol, blood pressure, boosts immunity and prevents ageing (Devi et al., 2014).

Natural antimicrobial compounds are secondary metabolites that can be found in plants, animals, and microorganisms. Plants, especially herbs and spices, are been given more attention in natural antimicrobial research. Microorganisms that have been used in food fermentation also produce different antimicrobial metabolites including organic acids, hydrogen peroxide, ethanol, and diacetyl in addition to bacteriocins. Products of animal origin such as tissues, milk and eggs contain different antimicrobial agents that are mainly in the form of peptides (polypeptides). In most cases natural antimicrobials are extracted and purified to be tested or applied to food products (Saeed A H et al.2013).

For thousands of years medicinal plants have played a significant role in the treatment of a wide range of medical conditions, including infectious diseases. Some naturally occurring chemical compounds serve as models for a large percentage clinically proven drugs, and many are now being re-assessed as antimicrobial agents (Mahady G B. et al. 2008).

2. Present Work:

For present study, different extracts and compounds isolated from the *S. cumini* (Bark) were taken. Plant extracts showed significant antibacterial and antifungal activity. Thus it was planned to screen the antimicrobial activity of extracts and compounds isolated from *S. cumini* (Bark).The following extracts and compounds were taken for the screening of antimicrobial activity.

Extracts:

For the present work we have taken hexane, benzene, chloroform, ethyl acetate, acetone, ethanol, and methanol fractions of the ethanol extract obtained from the study material (Bark) were screened for their antibacterial activity (Gautam et al.2012). Following listed gram negative and gram positive bacteria were taken for the activity.

Gram –ve

Salmonella typhi.

Escherichia coli

Klebsiella pneumoniae

Pseudomonas aeruginosa

Citrobacter freundt

Aeromonas hydrophilia

Gram +ve

Staphylococcus aureus

Bacillus subtilis

Listeria monocytogenes

Streptococcus pyogenes

Staphylococcus faecalis

Corynebacterium Hoffmannii

Fungi and yeast

Following fungi and yeast were taken for the screening:

Rhizopus nigricans

Candida krusei

Curvularialunata

Candida albicans

Aspergillus niger

3. Materials and Methods:

The extracts and compounds were tested for antibacterial and antifungal activity using Muller Hinton Agar and Sabouraud Dextrose Agar (SDA) plates respectively. Zone of inhibition was measured to assess the antibacterial and antifungal activity. This method depends on the diffusion of various extracts from a cavity through the solidified agar layer in such a way that the growth of microorganism inhibited around cavity containing the extracts and compounds. Growth of microorganism was spreaded uniformly on the surface of Muller Hinton agar with a glass spreader. Wells of 4mm diameter were created with sterile test tube and filled with 200 µl of extracts and compounds using micropipette. After addition the plates were pre-incubated at room temperature to allow diffusion and then incubated at 37⁰C for 24 hours. After incubation the diameter of zone of inhibition was measured in mm. The experiments were carried out in triplicate.

4. Results and Discussion:

Analysis of Antibacterial activity of various Extracts of *S. cumini* (Bark)

The results have shown that hexane extract showed poor activity against all the microorganisms. Whereas DCM extract gave significant zone of inhibition against *P. aureginosa*, *B. subtilis* and moderate with *S. typhimurium*. The ester extract gave significant zone of inhibition against *E. Coli*, *P. Aureginosa* and *S. typhimurium*. Ethanol and Methanol extract showed noticeable activity against *E. coli*, *B. subtilis*, *P. aureginosa* and *S. aureus*. The chloroform and acetone fractions showed lesser activity shown by the formation of smaller zone of inhibition against all the tested microbes.

The results were summarized in the table 1 and 2; fig. 1 and 2.

Analysis of Antifungal activity of various extracts of *S. cumini* (Bark)

All prepared extracts of *S. cumini* are recessive against *R. nigricans*. Hexane extract has showed poor activity against all fungal strains. DCM and ethyl acetate extracts has showed moderate activity against *C. albicans* where as ethanol and methanol extracts has indicated significant activity against *C. albicans* and *A. niger*.

The results were summarized in the table 1 and 2; fig. 1 and 2.

Experimental:

The antimicrobial activity was screened by filter paper disc method (C D lee *et al.* 1996; Paris A *et al.* 1996).

Requirements(formula ingredients)	Muller-Hinten Agar(g/litre)
Beef,heart,infusion form	300
Casein acid hydrolysate	17.5
Starch	1.5
Agar	17.5
Distilled water	1 Litre
Final pH(at 25 °C)7.3±0.2	
Potato Dextrose agar	
Potato infusion form	200
Dextrose	20
Distilled water	1 Liter
Final pH(at 25 °C)7.3±0.2	
Nutrient Broth	
Peptone	5.0
Beef extract	3.0
Distilled water	1 Litre

Procedure

1) Prepration of Inoculums:

Inoculums of bacteria were prepared in nutrient broth and fungi in Potato Dextrose Agar slant .They were inoculated and incubated for 48 hours in case of bacteria and 5 days in case of fungi.

2) Preparation of Seeded agar plates:

The Molten Hinton agar was poured in sterile petridish to get a depth of 4mm.The medium was left to solidify. There after it was seeded with respective test organisms. For the purpose of seeding,5 ml sterile water was added to each agar slant culture at fungi. The culture was scrupt to get suspension of fungi spore. A sterile cotton swab was dipped in the culture /suspension and lightly rubbed over the solidified medium. The plate was left for few minutes and then used for the test.

3) Preparation of the sample:

Approximately 10 mg of each sample to be tested was dissolved in 1 ml of the respective solvent.

4) Determiation of the activity:

4 mm disc of Whatmann filter paper No.42 were cut and sterilized. The filter paper disc were immersed in the solution of sample, after soaking, the disc was removed and left in a sterile petridish to permit the solvent to evaporate. After about 10 minutes the paper disc were transferred to the seeded agar plate. Near the periphery of the petridish 4 disc were kept on the seeded agar plate. In the center the fifth disc were also placed which was soaked with the standard solution. After the discs were transferred to the seeded plates the petridishes were incubated at 37°C for about 24 hours. At the end of incubation each plate was observed for the zone of inhibition. Each distinct inhibition zone was measured as diameter in millimeter. The experiment was performed in triplicate and average zone of inhibition was reported.

Table No. 1: Antibacterial activity of various extracts of *S.cumini* (Bark) in terms of mean zone of inhibition (mm).

Sr. No.	Extracts	Microorganism							
1	Hexane	07	03	04	06	02	05	04	05
2	DCM	09	14	05	10	08	04	03	06
3	CHCl ₃	05	11	03	08	08	12	14	06
4	Ethyl Acetate	20	16	11	18	14	12	09	08
5	Acetone	08	03	05	07	03	07	10	04
6	Ethanol	19	17	11	08	18	16	11	03
7	Methanol	20	10	12	14	17	22	08	04
8	Control	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ
9	Standard	30	25	26	28	26	28	26	26

Table No. 2: Antifungal activity of extracts of *S.cumini* (Bark) in terms of mean zone of inhibition (mm).

Sr. No.	Fungal Strains	Extracts								
		Hexane	DCM	CHCl ₃	Ethyl Acetate	Acetone	Ethanol	Methanol	Control	Standard
1.	<i>R. nigricans</i>	02	04	05	07	04	02	07	NZ	25
2.	<i>C. krusei</i>	04	07	06	08	02	04	07	NZ	20
3.	<i>C.lunata</i>	03	05	04	08	08	07	09	NZ	28
4.	<i>C. albicans</i>	06	14	03	10	04	14	12	NZ	30
5.	<i>A. niger</i>	03	05	08	14	06	11	12	NZ	26

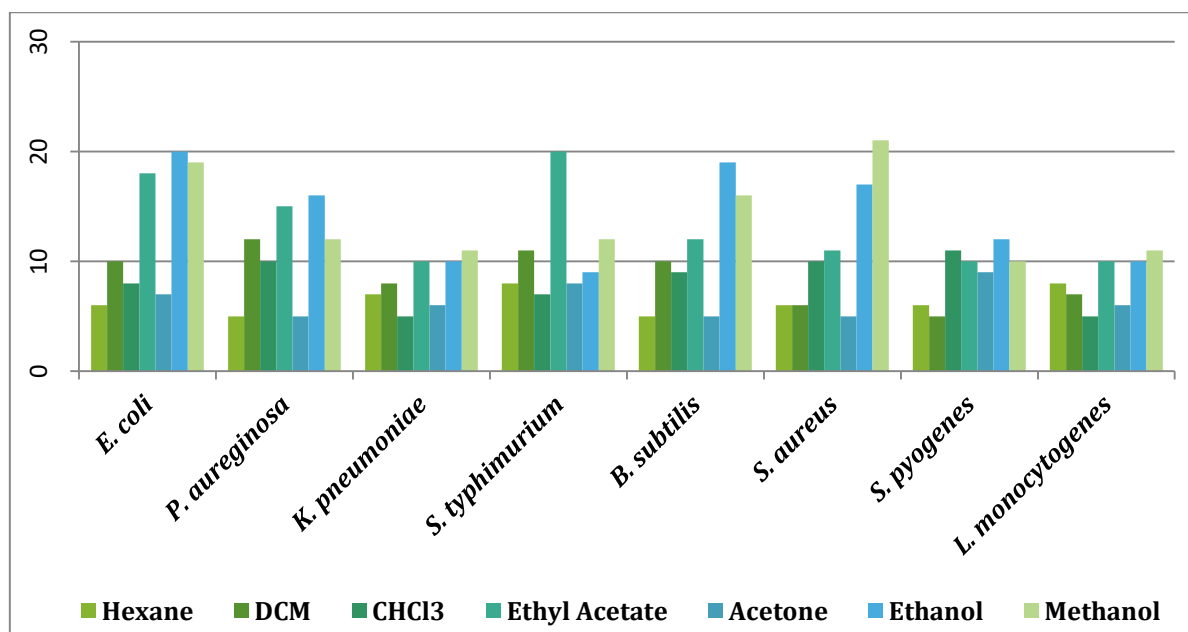


Fig. No. 1: Chart representing antibacterial activity of extracts of *S.cumini* (Bark)

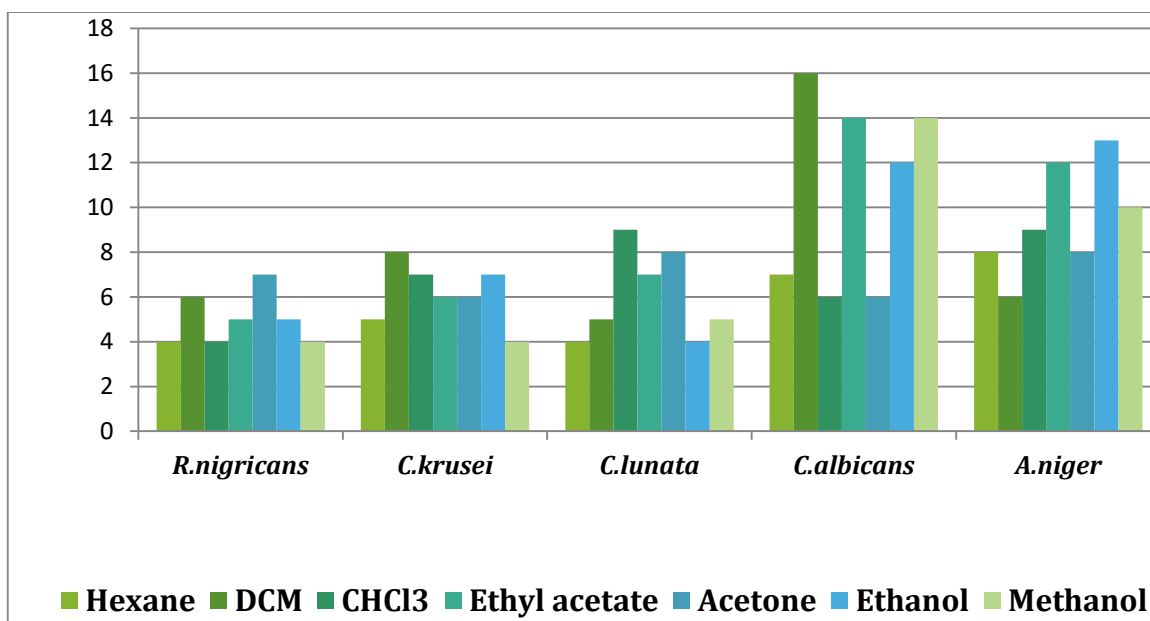


Fig. No. 2: Chart representing antifungal activity of extracts of *S.cumini* (Bark)

5. Conclusion:

Experimental studies have demonstrated its broad spectrum of antibacterial and antifungal activities against many of bacterial and fungal strains with different diameter zone of inhibition. The broad spectrum of antibacterial and antifungal activities of the plant extract, possibly due to the secondary metabolites such as flavonoids, tannins, phenolic compounds or saponins that were abundant on this plant. Therefore this study paves the way for further attention and research to identify the active ingredients responsible for the plant biological activity. So this study review the role of *S.cumini* (Bark) as therapeutic agents and it can be used in the treatment of infectious diseases caused by resistant microbes.

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