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Research Article

VARIATIONS IN ANTIMICROBIAL ACTIVITY OF *SYZYGIUM CUMINI* OF DIFFERENT GEOGRAPHICAL LOCATIONS

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ABSTRACT

The emergence of antibiotic resistance and the evolution of new strains of disease causing agents, are of highly concern to the global health community. Plants are potential source of antimicrobial agents. They have been used traditionally for prevention of infections caused by micro-organisms. Description of *Krimighana* herbs enumerated in Ayurveda classics is suggestive towards the importance of this group of medicine. *Jambu* (*Syzygium cumini* (L.) Skeels) is a member of Myrtaceae family. In Raja Nighantu it is mentioned that plant *Jambu* is having *Kriminashaka* property. It has been widely used medicine in the prevention of various ailments like cough, Dysentary, Diabetes, inflammation and ringworm. It is well established fact that geographical variations effects the potential and activity of medicinal herbs. Hence, the present study was undertaken to investigate *Syzygium cumini* procured from different geographical locations including Delhi, Rajasthan and Maharashtra for their potential activity against human infections caused by pathogens. Method The aqueous extract of *Syzygium cumini* of all the three areas was prepared. The activity of the plant extract was evaluated against nine bacterial pathogens and one fungal strain, which include *Staphyllococcus aureus, Streptococcus pyogenes, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Klebsiella pneumonia* and *Candida albicans*. The activity was carried out using Disk diffusion method. **Result and Conclusion:** All samples of *Syzygium cumini* showed potential antimicrobial activity against four pathogens including *Staphyllococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa* and *Candida albicans*. MIC was also evaluated against the tested pathogenic strains. The sample from Maharashtra showed MIC i.e. 80µg, 40µg, 80µg against *Staphyllococcus aureus*, *Streptococcus pyogenes* and *Candida albicans* respectively which is less as compare to sample from Rajasthan and Delhi. Region wise sample from Maharashtra showed good ZOI and MIC.

KEYWORDS: Antibiotic resistance, *Syzygium cumini*, Geographical variation.

INTRODUCTION

Infectious diseases are the significant cause of morbidity and mortality among the population, particularly in developing countries. Therefore, pharmaceutical companies have been forced to develop new antimicrobial drugs due to the regular emergence of microorganisms resistant to conventional antimicrobials drugs[1]. But in spite of development of newer generations of antimicrobials, antimicrobial drug resistance stresses the need for the development of new drugs. But these antimicrobial drugs (antibiotics) can affect the gut microbiota.[2]

The World Health Organization have drawn attention towards the threat to public health.[3]

The Plants have been a precious source of natural products for maintaining human health. Medicinal plants would be the best source to obtain a variety of drugs according to World Health Organization (WHO). About 80% of individuals from developed countries using traditional medicine, which has compounds obtained from medicinal plants.[4]

Ayurveda is a treasure of medicinal plants which are much useful in building human health care system and disease management In Ayurveda the description of Antimicrobials are enumerated under *Krimighna* and medicinal plants are said to have *Krimighna* potential[5]. There are various herbal preparations which are clinically used to cure infectious diseases. Plant-derived medicine are relatively safer than modern medicines and put forward many therapeutic benefits.[6]

Many types of naturally occurring antimicrobial compounds are presented in medicinal plants that can be effectively used against microbial infections.[7]

Syzygium cumini member of Myrtaceae family, is one of the medicinal plant which is rich in herbal active constituents which have many antimicrobial compounds. In *Raja Nighantu* it is mentioned that plant *Jambu* is having *Kriminashaka* property[8]. It has been widely used in variety of ailments like cough, Dysentary, Diabetes, inflammation and ringworm.[9]

Its biological efficiency is noticed as anti diabetic activity[10], anti-inflammatory activity[11], Hepatoprotective[12], antioxidant activity[13], antihistaminic activity[14] etc.

Phytochemical screening of extracts of *Syzygium cumini* revealed that seed contains alkaloids, amino acids, phytosterols, saponins, steroids, tannins and triterpenoids[15] etc.

In this study, we have investigated the antimicrobial activity of *Syzygium cumini* seeds from three different geographical locations including Delhi, Rajasthan and Maharashtra against *Staphyllococcus aureus, Streptococcus pyogenes, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Klebsiella pneumonia, Haemophyllus influenzae, Proteus* and *Candida albicans* using Disk Diffusion Technique.

MATERIAL AND METHOD

a) Collection of *Jambu* (*Syzygium cumini*) seeds, authentication and powder preparation.

Fresh fruits of *Jambu (Syzygium cumini)* were collected from natural habitat of Delhi, Rajasthan and Maharashtra. Fruits were washed, seeds were separated from pulp. The sample of seeds of *Jambu* was authenticated by NISCAIR, New Delhi (Authentication number was NISCAIR/RHMD/ Consult/2019/3503-04-3). Seeds were then shade dried and powdered using sieve (80no.) and stored in air tight container.

The coding of the samples were done as

S1- Sample of *Syzygium cumini* taken from *Delhi*

S2- Sample of *Syzygium cumini* taken from *Rajasthan*

S3- Sample of *Syzygium cumini* taken from *Maharashtra*

Vancomycin and Amikacin were taken as standard.

b) Preparation of Extract

Drug extractions of all samples were done in *Dravyaguna* and *Rasshastra* department of All India Institute of Ayurveda, New Delhi.

Twenty gram of powdered plant material was taken in 100ml distilled water in a conical flask. The mouth of conical flask was closed. It was than kept on magnetic shaker for six hours for continuous shaking and allowed it to stand for eighteen hours. Then the extract was filtered by using Whatman filter paper grade 1. The filtrate was evaporated using water bath at 100˚C. The dried extract powder was than collected.

Preparation of Disk

Following concentrations (in µg) of all extract were made from their stock solution:

Syzygium cumini (S1, S2 and S3) - 10, 20, 40, 80,160,320,640,1280. Inside biosafety cabinet, Disks were prepared by using the Disk diffusion method of Kirby and A.W.Bauer[16]. A 3mm Whatman filter paper disk was impregnated with the concentrations used.

Pathogen Strains

Strains of seven pathogens including Staphyllococcus aureus, Streptococcus pyogenes, Escherichia coli, Psudomonas aeruginosa, Salmonella typhi, Klebsiella pneumonia and Candida albicans were collected from the laboratory samples of Department of Microbiology, Kasturba Hospital, Delhi.

Disk Diffusion Method

Pathogenic strains were cultured on Blood Agar and Mac-Conkey agar and incubated for 24 hours. When the growth was seen, micro-organism was inoculated in Peptone water and incubated at 37˚C for two hours and this was used as inoculums. A sterile cotton swab was inserted into the microbial suspension and compressed against the wall of the test tube so as to remove the excess fluid. The surface of nutrient agar plate was inoculated with the cotton swab. To ensure that the growth is uniform, the swab is passed three times over the entire surface. The three nutrient agar plates were then allowed to absorb in incubator for one hour.

After one hour, disks of different concentrations of all the three samples were applied with the help of forceps on the plates in clockwise direction.

RESULT

The current study showed that all samples of plant *Syzygium cumini* exerted antimicrobial activity against four pathogenic strains including *Staphyllo-coccus aureus* (Fig.1,Fig.2,Fig.3), *Streptococcus pyogenes* (Fig.4,Fig.5,Fig.6), *Pseudomonas aeruginosa* (Fig.7,Fig.8,Fig.9) and *Candida albicans* (Fig.10, Fig.11). The antimicrobial activity was seen to be direct proportion to the concentration used. The effect of different concentrations of all samples of *Syzygium cumini* against selected micro-organisms is tabulated in table 1. The minimum inhibitory concentration (MIC) was presented in table 2.

Table1: Zone of Inhibition (±0.5mm) of all samples of *Syzygium cumini* on all test strains

Samples	10	20	40	80	160	320	640	1280
S. aureus								
S1	-	-	-	5	8	12	16	18
S2	-	-	-	-	4	8	8	14
S3	-	-	-	4	5	10	14	20
S.pyogenes								
S1	-	-	-	4	8	12	12	16
S2	-	-	-	4	4	4	8	12
S3	-	-	4	5	10	12	12	16
P.aeruginosa								
S1	-	-	-	-	-	-	4	8
S2	-	-	-	-	-	-	4	6
S3	-	-	-	-	-	-	6	10
C.albicans								
S1	-	-	-	8	10	16	16	18
S2	-	-	-	-	4	14	14	18
S3	-	-		5	5	12	16	20

S1- Sample of *Syzygium cumini* taken from *Delhi*, S2- Sample of *Syzygium cumini* taken from *Rajasthan*, S3- Sample of *Syzygium cumini* taken from *Maharashtra.* The sample S3 showed higher ZOI and the sample S2 showed lower ZOI.

Table 2: MIC of all samples of *Syzygium cumini* against *S. aureus, S. pyogenes, P. aeruginosa* and *C. albicans*

Micro-organisms	Samples	MIC
S. aureus	S1	80
	S2	160
	S3	80
S. pyogenes	S1	80
	S2	80
	S3	40
P. aeruginosa	S1	640
	S2	640
	S3	640
C. albicans	S1	80
	S2	160
	S3	80

S1- Sample of *Syzygium cumini* taken from *Delhi*, S2- Sample of *Syzygium cumini* taken from *Rajasthan*, S3 - Sample of *Syzygium cumini* taken from *Maharashtra*

Figures

Antimicrobial activity of *Syzygium cumini* against *Staphyllococcus aureus*

Figure 1: Showing activity of S1

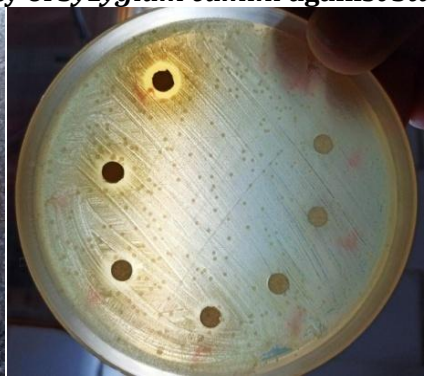

Figure 2: Showing activity of S2

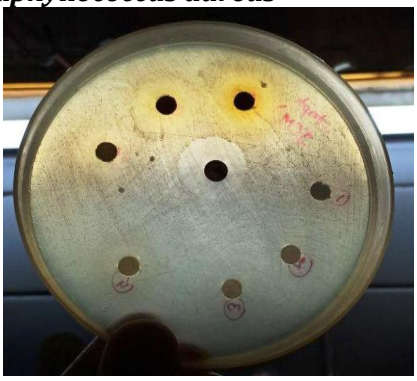

Figure 3: Showing activity of S3

Antimicrobial activity of *Syzygium cumini* against *Streptococcus pyogenes*


Figure 4: Showing activity of S1


Figure 5: Showing activity of S2

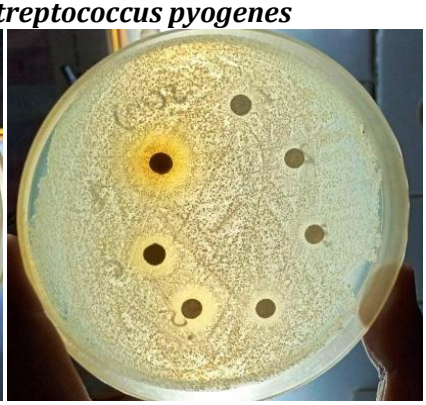
Figure 6: Showing activity of S3

Antimicrobial activity of *Syzygium cumini* on *Pseudomonas aeruginosa*


Figure 7: Showing activity of S1


Figure 8: Showing activity of S2


Figure 9: Showing activity of S3

Antimicrobial activity of *Syzygium cumini* against *Candida albicans*


Figure 10: Showing activity of S1

Figure 11: Showing activity of S2

The standard *Vancomycin* showed 20mm ZOI against *Staphyllococcus aureu*s, 12mm against the *Pseudomonas aeruginosa* and the standard Amikacin showed 20mm ZOI against *Streptococcus pyogenes.* No antimicrobial activity was exerted by all samples of *Syzygium cumini* against *Escherechia coli, Salmonella typhi*, *Klebsiella pneumonia, Haemophyllus influenzae* and *Proteus*.

DISCUSSION

Antibiotic resistance has created a problem to the healthcare sector in a large part of the world. This have increased a search for a new type of antimicrobial substances like plants as they contain a variety of phytoconstituents and bioactive compounds of known therapeutic properties.[17] The plant *Syzygium cumini* (Jambu) contains a large amount of secondary metabolites including alkaloids, flavonoids, terpenoids, steroids, tannins, saponins and reducing sugars.[18]

The change in geographical location associated with the climatic change can cause noticeable effect on the activities of medicinal and aromatic plants.[19]

Therefore, in the current study, we explored the seeds of the plant *Syzygium cumini* taken from three areas of India including *Delhi, Rajasthan* and

Maharashtra for their antimicrobial effectiveness against target pathogens that include *Staphyllococcus aureus, Streptococcus pyogenes, Escherichia coli, Psudomonas aeruginosa, Salmonella typhi, Klebsiella pneumonia* and *Candida albicans.*

There previous study done by *Anupam kumar et al* using aqueous extract of *Syzygium cumini* seeds showed that seed exhibited antimicrobial activity against *Staphyllococcus aureus* and *Escherichia coli* with ZOI 4.6mm and 7.3mm respectively[20] whereas our study showed 20mm ZOI against *Staphyllococcus aureus* and no activity was found against *Escherichia coli*.

The present study showed significant antimicrobial effect of all samples against four pathogens including Staphyllococcus aureus, Streptococcus pyogenes, Psudomonas aeruginosa and Candida albicans. The effective activity of all three samples was measured through ZOI and MIC against the tested microbial strains. The MIC was recorded for only those pathogens which have shown the ZOI. However, all samples have shown difference in their ZOI at different concentrations. The ZOI against Staphyllococcus aureus ranging from 5–20)mm. It was about to range 4-16mm against Streptococcus pyogenes whereas 6-10mm against Pseudomonas aeruginosa and 5-20mm against Candida albicans. The MIC measured against the tested pathogenic strains also varies. The sample from Maharashtra showed MIC i.e. 80µg, 40µg, 80µg against Staphyllococcus aureus, Streptococcus pyogenes and Candida albicans respectively which is less as compare to sample from Rajasthan and Delhi. Region wise sample from Maharashtra showed good ZOI and MIC (Table 1 and 2).

The antimicrobial potential of *Syzygium cumini* is contributed by a variety of secondary metabolites including alkaloids, flavonoids, terpenoids, steroids, tannins, saponins and reducing sugars[18].

Therefore we may infer that *Syzygium cumini* posseses good antimicrobial activity which may be due to presence of various bioactive compounds. The results also indicated that geographical conditions could lead to the variations in bioactivities of the plants as the sample of *Syzygium cumini* collected from *Maharashtra* has shown better results as compared to the samples of Delhi and Rajasthan.

CONCLUSION

It was shown from the current study that seeds of *Syzygium cumini* exhibited synergistic antimicrobial effect against the test pathogens. It contributed the potential activity against *Staphyllococcus aureus, Streptococcus pyogenes* and *Candida albicans* than *Pseudomonas aeruginosa*. Moreover, it was evaluated that among all samples, sample from *Maharashtra* have shown good ZOI as the concentration increases and MIC was found to be at lower than other samples. These results suggested the importance of growing environment selection for better use of this plant. The diverse geographic factors may affect the phyto-constituents in different *Syzygium cumini* samples.

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