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Research Article

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Antibacterial activity of *Syzygium cumini* (Java plum) seed extract against wound infection causing pathogens

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Abstract

Bioactive compounds from the natural resources have the potential to treat wide range of bacterial pathogens causing deadly infections. Anti-inflammatory, anti-diabetic and anti-cancer activity of the Jamun seed were well demonstrated. This present study evaluates the antibacterial activity and phytochemical screening of *Syzygium cumini* (Jamun seed) extract against the wound pathogens (*Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Proteus mirabilis*). Different concentrations of the Jamun seed extract was used against wound infection causing Bacteria and its zone of inhibition was measured. Among the three solvents (Methanol, Ethanol and Acetone) used in this study methanol extract of *Syzygium cumini* showed maximum activity against *Escherichia coli* (23mm) followed by *Enterococcus faecalis* (18mm) and *Staphylococcus aureus*(17mm). Phytochemical screening of Jamun extract reveals the presence of Alkaloids, Flavonoids, Saponins, Steroids, Tannins, Triterpenoids. These results showed that the Jamun seed extract had selective bactericidal activity against wound pathogens and also the richest source of antimicrobial proteins. It may be used as the drug against wound infection causing pathogens after the pharmacological studies.

Keywords: Antibacterial, Phytochemical, Wound infection, MIC

Introduction

Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Ncube *et al*., 2008). From ancient times, medicinal herbs have been use in one form or another, under indigenous systems of medicine like Ayurveda, Sidha and Unani. Herbal medicines are promising choice over modern synthetic drugs as they show minimum or no side effects and are considered to be safe (Modi *et al*., 2010). In developing countries basically people belong to rural areas want the cheaper medicines to cure diseases and infections and they are mainly depended on herbal medicines for their proper health (Faria, 2011).

Natural products have been a significant source of commercial medicines and drug leads. Screening of crude plant extracts paves the way for discovery of novel bioactive compounds, and elucidation of their structures can open the door for new synthetic preparations (Colegate and Molyneux, 2008). According to WHO, 80% of the world's inhabitant's problem should treated by medicinal herbal drug their primary health care (Dubey *et al*., 2004). *Syzygium cumini* L belongs to family Myrtaceae which is known by different names like jamun, java plum, jambul, and Indian blackberry.

S.cumini seeds have hypoglycaemic, anti-inflammatory, antipyretic, psychopharma-cological, hypolipidaemic and antioxidant activities. It is reported that the jamun seed extracts given to animals with 5 g/Kg body weight was more effective than glibenclamide, an anti-diabetic or hypoglycemic drug mostly given orally in case of diabetes type-2. It is also reported that glibenclamide may inhibit glucoamylase in vivo. Thus, it controls the degradation of glycogen and maintains glucose level in the blood. Reports are available that a decoction of the dry leaves of the *Syzygium cumini* gives hypoglycemic effect (Coimbra, *et al*., 1992).The seed of the fruit is used in various alternative healing systems and seed powder is active against various species of microbes like *E. coli*, *Bacillus subtilis*, *Streptococcus* and *Staphylococcus* (Banerjee, 2011). Pharmacologically proved to possess hypoglycemic, antibacterial, anti-HIV activity and antidiarrhea effect. Phytochemical extracts of *S. cumini* have been reported also having antioxidant properties and increased scavenging activity has been confirmed against reactive oxygen species (ROS) (Ruan, 2008). *S. cumini* extracts have potential to develop as green drug to treat various diseases and infections. Phytochemicals are naturally occurring in the medicinal plants, leaves, vegetables and roots that have defense mechanism and protect from various diseases (Krishnaiah *et al*., 2007).

Materials and Methods

Procurement of test cultures

The wound infection causing pathogens *viz Escherichia coli, Proteus mirabilis., Klebsiella pneumoniae, Staphylococcus aureus., Enterococcus faecalis* were procured from the Department of Microbiology, RMMCH, Annamalai university.

Sample collection

Syzygium cumini were procured from the local market. The spices were washed with 70% alcohol and then rinsed with sterilized distilled water, shade dried for 10-12 days and powdered using mechanical grinder and then stored in air tight containers for further use. Three extracts viz., ethanol, methanol and acetone were prepared.

Preparation of *Syzygium cumini* seed extracts

Preparation of extract:

10 grams of seed powder of *Syzygium cumini* were soaked separately in 100ml of Methanol, Ethanol, Acetone in a beaker separately. The beaker was closed with aluminum foil and small

holes were created on it. It was then kept at room temperature for 5 days and filtered through Whatman No.1 filter paper. The filtrate was poured in petri dish and evaporated at room temperature for 2-3 days till the volume was reduced to one fourth of the original volume of the solvent and stored at 4 c in air tight bottles (Harborne, 1973).

Determination of antibacterial activity of seed powder

Antibacterial activity of methanol, ethanol, and acetone extract of the *Syzygium cumini* powder was evaluated separately by agar well diffusion method (Aida *et al*., 2001).The test bacterial strain was inoculated into Mueller-Hinton broth and incubated at 37◦ c for 24 hours. After incubation a sterile cotton swab was immersed inthe bacterial suspension and swabbed aseptically on the surface of Muller-Hinton agar medium and allowed to dry for about 3 minutes. Well of 6mm diameter was punched into the agar medium and filled with 100 micro litre of crude seed(*Syzygium cumini*) extracts, 125 micro litre and 150 micro litre of different concentrations were used.

Gentamycin (50 mg/ml) was used as positive control and 10% dimethyl sulfoxide (DMSO) was used as a negative control. The plates were incubated in an upright position at 37 overnight in an incubator. Antibacterial activity was detected by measuring the zone of inhibition around each well, excluding the diameter of the well in mm as low activity (<=6 mm) moderateactivity (7-10mm) high activity (11-15mm) very active at (>= 16mm) and no activity (-) (Praveen *et al*., 2010).

Minimum inhibitory concentration of methanolic extract against pathogenic strains.

Culture; Overnight Muller Hinton Broth cultured of *Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Escherichia coli, Proteus vulgaris,*at 37˚C were prepared. The culture was adjusted to obtain turbidity comparable to that turbidity of MIC, Farland 0.5 standard (Hossain MD) and then further diluted 1:200 in Muller Hinton Broth. The inoculation thus prepared and expected to obtain 105 or 106 C.F.U/ml.

Muller-Hinton broth was prepared and poured into 7 tubes containing 3ml and autoclaved.A loopful of culture is then inoculated at test tubes except for media control. Seed extract solutions are added in increasing concentration in 5 test tubes (50, 100, 150, 200, 250 µl).The test tubes are then incubated at 37˚C for 24 hrs.

The tubes were examined for growth and were determined the MIC of tested antibiotics, which is bacteriostatic for the test organisam. The tubes were examined for visible growth (cloudy) and was recorded growth as (+) and no growth as (-).

Phytochemical screening

The seed extracts of *Syzygium cumini* were analysed for the presence of alkaloids, glycosides, triterpenoids, steroids, saponins, flavonoids, tannins and carbohydrates according to standard methods (Harborne 1998), (Kokate 2001).

Test for Alkaloids:

2 ml of dilute hydrochloric acid was added to the 5 ml of extract then treated with Dragondroff'sreagent, appearance of an orange brown precipitate showed the presence of alkaloids.

Test for Glycosides:

The extract was hydrolysed with dilute hydrochloric acid for few hours on a water bath.1 ml of pyridine and a few drops of sodium nitroprusside solution were added. Then 2-3 drops of dilute NaOH was mixed. Pink colour produced which turn into red indicated presence of glycosides.

Test for Triterpenoids:

About 5 ml of extract was mixed in 2 ml of chloroform; 2 ml of acetic anhydride and a few drops of conc. H2SO4 was added. Reddish violet colour indicated the presence of triterpenoids.

Test for Steroids:

10ml of chloroform was mixed with 2ml of extracts and conc. H2SO4 was added to form lower layer. A reddish yellow colour at the interface was an indicative of the presence of steroidal ring.

Test for Saponins:

15 ml of distilled water was added to the extract and shaken vigorously until formation of a stable persistent froth which indicates presence of saponins.

Test for Flavonoids:

Few drops of dilute NaOH was mixed with 2 ml of extract. A yellow solution that turns colourless showed the presence of flavonoids.

Test for Tannins:

In a test tube containing little quantity of extract few drops of 1 % lead acetate were added. Yellow precipitate appeared it showed the presence of tannins.

Test for Carbohydrates:

The small portion of extract was mixed with 2ml of Molisch's reagent and the mixture was shaken properly. After that 2ml of concentrated H2SO4 was poured carefully along the side of the test tube. Violet ring at the interphase was not formed which indicates absence of carbohydrate.

Results and Discussion

Antibacterial Activity of *Syzygium cumini* Against Bacterial Test Cultures:

In this present study to evaluate the antibacterial activity of *Syzygium cumini* against wound infection pathogens were represented in **table 1.** Maximum zone of inhibition was noted against *Escherichia coli* (17mm), *Staphylococcus aureus* (16mm), and *Enterococcus faecalis* (14mm) in diameter at ethanolic extract of 150µl concentration. Ethanol extract of *S. cumini* bark was reported to inhibit various isolates of V. cholerae (Sharma *et al*., 2009) with MIC values much higher (2.5-20 mg/mL) than other work. This indicates seeds to have better anti-vibrio activity than bark of same plant.

Table 1: Ethanolic extract of *Syzygium cumini* seed against some Wound infection pathogens.

S.No	Pathogens	Zone of inhibition (mm)			Positive Control (Gentamycin)
		100µl	125µl	150µl	
1	*Enterococcus faecalis*	11	12	**14**	23
2	*Escherichia coli*	12	14	**17**	26
3	*Klebsiella pneumoniae*	10	12	14	25
4	*Staphylococcus aureus*	10	12	**16**	23
5	*Proteus mirabilis*	11	13	14	25

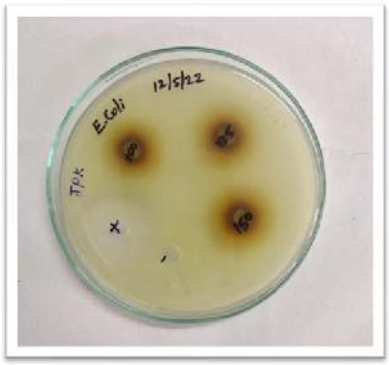

Fig:1Methanolic extract of *Syzygium cumini* against *Escherichia coli*

The zones of inhibitions were produced by both the aqueous and methanol extracts against all the test organisms. Methanol extracts were more active than the aqueous extract against all the microorganisms (Fridous *et al*., 1990). Similarly antibacterial activity of *Syzygium cumini* against wound infection pathogens were represented in **table 2.** Maximum zone of inhibition was noted against *Escherichia coli* (23mm), *Staphylococcus aureus* (18mm), and *Enterococcus faecalis*., (17mm) in diameter at methanolic extract of 150µl concentration.The antibacterial activity of *Syzygium cumini* against wound infection pathogens were represented in **table 3.** Maximum zone of inhibition was noted against *Escherichia coli* (16mm), *Staphylococcus aureus* (15mm), and *Enterococcus faecalis* (14mm) in diameter at acetone extract of 150µl concentration.

Table 2: Methanolic extract of *Syzygium cumini* seed against some Wound infection pathogens.

S.No	**Pathogens**	**Zone of inhibition (mm)**			**Positive Contol** (Gentamycin)
		100µl	**125µl**	**150µl**	
1	*Enterococcus faecalis*	12	14	**17**	23
2	*Escherichia coli*	16	19	**23**	26
3	*Klebsiella pneumoniae*	11	13	15	25
4	*Staphylococcus aureus*	12	14	**18**	23
5	*Proteus mirabilis*	11	12	15	25

Table 3: Acetone extract of *Syzygium cumini* seed against some Wound infection pathogens

S.No	**Pathogens**	**Zone of inhibition (mm)**			**Positive Control (**Gentamycin**)**
		100µl	**125µl**	**150µl**	
1	*Enterococcus faecalis*	10	12	**14**	23
2	*Escherichia coli*	11	13	**16**	26
3	*Klebsiella pneumoniae*	9	11	13	25
4	*Staphylococcus aureus*	10	11	**15**	23
5	*Proteus mirabilis*	8	12	13	25

The minimum inhibitory concentration of *syzgium cumini* seed methanolic extract against wound infection pathogens were represented in **table 4**. Minimum inhibitory concentration was noted against *Escherichia coli* 150µl, *Enterococcus faecalis* 200 µl, *Staphylococcus aureus* 200 µl. The analysis of minimum inhibitory concentration was by agar dilution method to determine the antibacterial activity of *Syzygium cumini* seed extract against *Escherichia coli*.Talaro,2008 have reported that the MIC values of extracts against susceptible organisms ranged from 154-656 µg/mL. Both the extracts being active against gram- positive and gram negative bacteria can be labeled as having a broad spectrum of antibacterial activity.

Table 4: Minimum Inhibitory Concentration of Methanolic Extract Against Wound infection pathogens

Concentration (µl)	Turbidity				
	Enterococcus faecalis	***Escherichia coli***	***Klebsiella pneumoniae***	***Staphylococ cus aureus***	***Proteus mirabilis***
50	Turbid	Turbid	Turbid	Turbid	Turbid
100	Turbid	Turbid	Turbid	Turbid	Turbid
150	Turbid	**No visible turbidity**	Turbid	Turbid	Turbid
200	**No visible turbidity**	No visible turbidity	Turbid	**No visible turbidity**	Turbid
250	No visible turbidity	No visible turbidity	Turbid	No visible turbidity	Turbid
Contol	No visible turbidity	No visible turbidity	No visible turbidity	No visible turbidity	No visible turbidity

Phytochemical analysis

Preliminary phytochemical studies revealed the presence of flavonoids, alkaloids, glycosides, steroids, phenols, saponins, terpenoid, cardiac glycosides and tannins as the chemical class present in the extracts. The extracts showed inhibitory activity against clinical isolates of the gram negative bacteria such as *Salmonella enteritidis*, *Salmonella typhi*, *Salmonella typhi* A, *Salmonella paratyphi A*, *Salmonella paratyphi B*, *Pseudomonas aeruginosa* and *Escherichia coli*, gram positive bacteria are *Bacillus subtilis*, and *Staphylococcus aureus* (Shyamala Gowri and K. Vasantha, 2010).The results of phytochemical analysis are given in the **Table 5**. It revealed the presence of alkaloids, glycosides, triterpenoids, steroids, saponins, flavonoids, tannins except carbohydrates in the methanolic extract of *Syzygium cumini* seed showed the results of phytochemical analysis. Studies on them could lead to finding of novel drugs for effective treatment of various diseases caused by commensals and pathogens. We have chosen one of the most commonly used fruit *Syzygium cumini* for the present study to screen and analyze the phytochemical and antimicrobial properties they posses. The study revealed that *Syzygium cumini* extracts contains rich availability of carbohydrates, phenols, flavonoids and tannins as their secondary metabolites. (Shylaja *et al*., 2011).

Table 5: Phytochemical screening of methanolic extract of *Syzygium cumini* seed

Phytochemicals	Results
Alkaloids	+
Glycosides	+
Triterpinoids	+
Steroids	+
Saponins	++
Flavonoids	++
Tannins	+
Carbohydrates	-

+ = present, ++ = Moderately present, - = absent.

Conclusion

In this study, we have evaluated the Antibacterial activity, and MIC, phytochemical potential of the extracts obtained from the seeds of *S. cumini*. Among the three different extract tested, methanol extract exhibited maximum activity towards all the tested wound infection causing pathogens. The methanol seed extract of *S. cumini* was screened for the presence of phytochemical compounds.

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