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Research Article

ANTIBACTERIAL ACTIVITY OF UNDERUTILIZED FRUITS OF JAMUN (*SYZYGIUM CUMINI* L. SKEELS)

PRAKASH R. PATEL[1*] AND T. V. RAMANA RAO[2]

[1]Agharkar Research Institute, G. G. Agarkar Road, Pune, Maharashtra 411004, [2]Department of Biosciences, Sardar Patel University, Vallabh Vidyanagar, Gujarat 388120, India. *Email: prakashpatel@inbox.com

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ABSTRACT

There is a worldwide interest in identifying antibacterial compounds, especially from underutilized fruits against the increasing resistance of various disease causing organisms. The fruits of *Syzygium cumini* known to possess high medicinal value have been evaluated for its antibacterial activity against some gram positive and gram negative bacterial strains. Zone of inhibition were obtained against all bacterial strains tested except for *Micrococcus luteus* against ethyl acetate fractions and *Salmonella paratyphi* using diethyl ether and ethyl acetate fractions. High zone of inhibition was obtained against *Bacillus cereus* using diethyl ether extract. Lowest minimum inhibitory concentration value of 0.25 mg/ml of diethyl ether extract of preripened fruits was effective against *Bacillus cereus*. The activity of the extracts varied along with the fruits maturity, signifying the role of maturity indices in accumulation of bioactive compounds. Hence, the study revealed the antibacterial potential of Jamun fruit, which are underutilized possess rich bioactive compounds of medicinal potential to be exploited for the benefit of humankind.

Keywords: Antibacterial, Fruit, Jamun, *Syzygium cumini* L. Skeels, Underutilized

INTRODUCTION

Since the dawn of human civilization, plants have been a valuable source of natural products for maintaining human health. To ensure availability of drugs for the burgeoning populations search for pharmacologically active compounds from plant sources have been emphasized for its use in sustainable manner from the available natural sources. These natural plant products are known to be chemically balanced, effective and least injurious with none or reduced side effects as compared to synthetic medicines. Numerous bioactive compounds have been identified/ isolated from plant sources and introduced into clinical medicine, but over a period these drugs becomes less effective due to the increasing resistance of various disease causing organisms. Thus, the problem of resistance in disease causing organisms is growing widely and the outlook for the use of effective drugs against them in the future is also uncertain.

It has been well recognized that consumption of large amounts of fresh fruits and vegetables can bring substantial health benefits.[1] In India due to the diversity in climate, soil, altitudes and other eco-geographical conditions, rich resource of wild/ underutilized fruits are available in this region. These underutilized fruits have never demanded attention of the researchers but have chiefly served as a natural source of treatment for curing various diseases and ailments of the tribals/ local inhabitants.[2] Some studies on underutilized fruits have claimed them to be superior sources of nutrients and medicine over other commercially used. Hence, these underutilized fruits provide unlimited opportunities for screening of new drugs as they are known to possess an array of chemical diversity, which needs to be investigated. Hence the present study has been aimed to understand the antibacterial activity of *Syzygium cumini* fruits, which are underutilized.

Jamun (*Syzygium cumini* L. Skeels) is an important minor fruit of Indian origin commonly known as Black plum, found growing widely in different agro-climatic conditions. The fruits have been attributed to possess several medicinal properties in the Indian folklore medicine system. The fruits are used for curing diarrhea and are also used as a general tonic for the liver.[3] In addition, several other medicinal uses have been attributed to this fruits such as, it enriches the blood; strengthens teeth and gums; useful astringent in bilious diarrhea; good gargle for sore throat; good lotion for ringworm etc.[4] The plant have been reported to possess antioxidant and free radical scavenging activities,[5,6] antibacterial,[7-12] antifungal,[13,14] antidiabetic[15] and anti-inflammatory[16] activities. Thus, the plant is medicinally importance, but the fruits of Jamun have not been given due consideration to understand the antibacterial property. Hence the present study.

MATERIALS AND METHODS

Plant material: The fresh fruit samples of *Syzygium cumini* L. Skeels were collected during February - April, 2009 from the vicinity of Vallabh Vidyanagar, Gujarat, India at their sequential stages of growth and ripening. The fruits were cut opened, pulp of the fruits were separated, dried at room temperature, grounded to powder and finally stored in air tight containers until further use.

Sample extraction: The infusion extraction method given by Houghton and Raman was used.[17] Extraction was initiated using non polar solvent like diethyl ether followed by ethyl acetate, acetone, methanol and water. The resulting extracts were concentrated by drying them at room temperature and finally stored in refrigerator (4°C) until further use.

Bacterial cultures: To understand the antibacterial activity, the microbial pure cultures obtained from MTCC (Microbial type culture collection, Chandigarh, India) were used for the present study. Four gram positive bacterial cultures namely - MTCC-430 *Bacillus cereus* (BC), MTCC-121 *Bacillus subtilis* (BS), MTCC-106 *Micrococcus luteus* (ML), MTCC-435 *Staphylococcus epidermidis* (SE) and four gram negative bacterial cultures namely - MTCC-443 *Escherichia coli* (EC), MTCC-109 *Klebsiella pneumoniae* (KP), MTCC-735 *Salmonella paratyphi* (SP), MTCC-734 *Salmonella typhi* (ST) were used for the present study.

Zone of Inhibition (ZI): The antibacterial activity was screened by using agar well diffusion method.[18] All the bacterial cultures used were grown on nutrient agar medium (pH 7.4) at 37°C. A 0.5 Mc Farland turbidity standard was used to measure the density of bacterial cells.[19] Antibiotics such as Ciprofloxacin and Doxycycline (20 µg/ml) were used as positive controls, while 100 and 50 % DMSO were used as negative controls. The diameter of the inhibitory zone was measured in mm. All the bioassays were carried out in triplicate to minimize the error.

Minimum inhibitory concentration (MIC): The extracts that gave an inhibition zone of 10 mm or more, were evaluated for their MIC values. Serial broth dilution method was used to prepare dilutions of extracts in range of 8 mg/ml to 0.250 mg/ml. Finally the presence of live bacterial population was determined by appearance of red colour, while collourless in case of dead bacterial population, using 2, 3, 5-triphenly tetrazolium chloride test.[20] The solutions containing

DMSO and nutrient broth were used as controls. The MIC values were carried out in three replicates to confirm the activity.

RESULTS

Zone of Inhibition (ZI)

Among the various extracts used for screening the antibacterial activity of Jamun fruit against some selected bacterial strains, superior activity was measured when diethyl ether extracts of mature and preripened fruit were used, which resulted in 15 and 20 mm zones respectively against *Bacillus cereus* (Table 1), followed by the ripened fruit against *Salmonella typhi* (12 mm). Moreover, good to moderate activity against *Bacillus cereus* was exhibited by the extracts of young, premature and ripened fruit, with the Zone of Inhibition measuring 10, 13 and 13 mm respectively (Table 1). High inhibition percentage was also recorded against bacterial strain *Bacillus cereus*, followed by *Salmonella typhi*, *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus epidermidis* and *Klebsiella pneumoniae*, while *Salmonella paratyphi* was found to be highly resistant, as it exhibited no inhibition zone against the diethyl extracts of all developmental stages of Jamun fruit. Furthermore, ethyl ether and acetone extracts exhibited moderate to less activity against most of the bacterial strains used (Table 1).

The methanolic extract exhibited good zone of inhibition against *Micrococcus luteus* using young (12 mm), premature (11 mm), mature (12 mm) and ripened fruit (15mm). The young and ripened fruit also showed good activity against *Salmonella typhi*, while premature fruit extract showed good activity against *Salmonella paratyphi* (Table 1). Moderate to less activity was monitored against *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli* and *Klebsiella pneumoniae* using methanolic extract of the Jamun fruit. In contrast, water extract of the ripened Jamun fruit showed good activity against *Salmonella typhi* (13 mm), *Micrococcus luteus* (12 mm), *Staphylococcus epidermidis* (10 mm) and *Salmonella paratyphi* (10 mm), while other bacterial strains exhibited moderate to less activity using water extract (Table 1).

Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration was checked to observe the dosage required to inhibit the growth of bacterial organism. The fruit of Jamun restricted the growth of *Bacillus cereus* with a low dosage of 0.25 mg/ml using diethyl ether extract of the preripened fruit (Table 2). Methanolic extract of both preripened and ripened fruit inhibited the growth of *Staphylococcus epidermidis* and *Micrococcus luteus* respectively at 1 mg/ml, while 2 mg/ml methanolic extract of mature fruit inhibited the growth of *Micrococcus luteus*. Moreover, diethyl ether extracts of premature, mature and ripened fruit, methanolic extract of young fruit and water extract of ripened fruit inhibited the growth of *Bacillus cereus*, *Staphylococcus epidermidis* and *Micrococcus luteus* respectively at 4 mg/ml. Furthermore, methanol extract of young fruit and water extract of ripened fruit inhibited the growth of *Micrococcus luteus* and *Salmonella typhi* respectively with a minimum inhibitory concentration of 8 mg/ml (Table 2).

Table 1: Zone of Inhibition obtained using various fruit extracts of *Syzygium cumini* at its sequential stages of growth and ripening

Extracts used	Stages of fruit growth and ripening	Zone of Inhibition (mm)							
		Gram $^{+ve}$ Bacteria				Gram $^{-ve}$ Bacteria			
		BC	BS	ML	SE	EC	KP	SP	ST
Diethyl ether	Young	10	9	3	6	7	1	-	7
	Premature	13	5	2	4	-	2	-	8
	Mature	15	4	3	7	2	5	-	6
	Preripened	20	3	5	4	9	3	-	7
	Ripened	13	4	6	4	6	2	-	12
Ethyl acetate	Young	4	1	-	4	1	2	-	3
	Premature	2	1	-	-	5	2	-	2
	Mature	5	2	-	2	3	2	-	2
	Preripened	2	4	5	2	5	-	4	5
	Ripened	4	3	3	4	4	-	5	5
Acetone	Young	2	5	1	1	3	3	5	3
	Premature	7	3	4	4	4	3	6	3
	Mature	7	4	5	3	3	3	9	2
	Preripened	4	6	6	4	4	7	7	3
	Ripened	2	3	9	1	5	5	6	5
Methanol	Young	4	9	12	13	7	5	8	10
	Premature	8	8	11	11	7	6	11	8
	Mature	4	7	12	7	6	3	9	9
	Preripened	4	6	8	15	9	3	4	8
	Ripened	9	9	15	9	9	9	9	11
Water	Young	9	4	6	5	3	4	5	5
	Premature	9	6	4	7	4	4	8	4
	Mature	5	5	7	8	3	4	8	5
	Preripened	3	5	5	9	6	3	8	5
	Ripened	5	6	12	10	9	5	10	13

BC – *Bacillus cereus*

BS – *Bacillus subtilis*

EC – *Escherichia coli*

KP – *Klebsiella pneumonia*

ML – *Micrococcus luteus*

SE – *Staphylococcus epidermidis*

SP – *Salmonella paratyphi*

ST – *Salmonella typhi*

Table 2: Minimum inhibitory concentration (MIC) using different extracts of *Syzygium cumini* fruit on some selected bacterial strains

Stage of fruit growth and ripening	Extract used	Bacterial strains	MIC (mg/ml)
Young	Diethyl ether	BC	-
	Methanol	ML	8
	Methanol	SE	4
	Methanol	ST	-
Premature	Diethyl ether	BC	4
	Methanol	ML	-
	Methanol	SE	-
	Methanol	SP	-
Mature	Diethyl ether	BC	4
	Methanol	ML	2
Preripened	Di-ethyl ether	BC	0.25
	Methanol	SE	1
Ripened	Diethyl ether	BC	4
	Methanol	ML	1
	Methanol	ST	-
	Water	ML	4
	Water	SE	-
	Water	SP	-
	Water	ST	8

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DISCUSSIONS

Mostly the availability of active substances is dependent on the type of the extraction solvent used. In the present study we found that the diethyl ether extracts gave high percentage of inhibition against the organisms tested followed by methanol, water, acetone and ethyl acetate fractions. The differences in the activity of vivid solvents have been reported earlier.[21,22] In Jamun, Shaikh *et al*. have reported that ethanolic extracts of Jamun inhibit both gram positive and gram-negative organisms.[23] While, Bhuiyan *et al*. has obtained antibacterial activity in methanol and ethyl-acetate extracts of Jamun seeds.[7] Besides, the essential oil of Jamun leaves are credited to obtain good antibacterial properties.[12]

A study by Bagchi *et al*., however, has shown considerable activity of *S. cuminii* against Gram-positive and Gram negative bacteria and fungi.[8] Bhuiyan *et al*. has obtained good antibacterial activity against five gram positive and nine gram-negative bacterial strains.[7] According to Ahmad and Beg the gram-positive bacteria are considered to be more sensitive as compared to gram-negative because of the differences in their cell wall structures.[10]

Rajakaruna *et al*. have reported photoactivity of *S. cumini*, which produced large zones of inhibition against both *S. aureus* and *B. subtilis*.[11] While, Nascimento *et al*. obtained promising antibacterial activity of Jamun against *Klebsiella pneumoniae*.[9] This photoactivity observed by *S. cumini* is thought to be due to the presence of monoterpene aldehydes.[24] Besides, the fruits have been reported to possess other bioactive compounds like like citric, mallic and gallic acid.[25] Other commonly referred as phytochemicals such as anthocyanins, alkaloids, carotenoids, flavonoids, polyphenols and tannins that are present within the fruits are also known to be effective and plays an active role as antibacterial substances against a wide array of infectious agents.[26,27]

Thus the study helps us to understand that these underutilized fruits have a great potential for antibacterial action. Besides, the maturity indices also play an important role in accumulation of these bioactive compounds. Although a large number of natural products have been approved as new antibacterial drugs, still there is an urgent need to identify more novel substances that are active towards pathogens of high resistance.

REFERENCES

1. World Cancer Research Fund. Food nutrition and the prevention of cancer. World Cancer Research Fund and American Institute for Cancer Research. Washington DC. 1997.
2. Rao MR, Palada MC, Becker BN. Medicinal and aromatic plants in agroforestry systems. Agrofor Syst 2004; 61: 107–122.
3. Kirtikar KR, Basu BD. Indian medicinal plants. Vol. 2., Intl. Books Distrib., Dehradun, India. 2006; pp. 1497.
4. Warrier PK, Nambiar VPK, Ramankutty C. Indian Medicinal Plants. Vol. 5. Orient Longman Ltd., Hyderabad, India. 1996; pp. 225-228.
5. Rekha N, Balaji R, Deecaraman M. Effect of aqueous extract of *Syzygium cumini* pulp on antioxidant defense system in Streptozotocin induced diabetic rat. Iranian J Pharm Therap 2008; 7(2): 137-145.
6. Zhang LL, Lin YM. Antioxidant tannins from *Syzygium cumini* fruit. African J Biotech 2009; 8(10): 2301-2309.
7. Bhuiyan MSA, Mia MY, Rashid MA. Antibacterial principles of the seeds of *Eugenia jambolana.* Bangladesh J Bot 1996; 25(2): 239-241.
8. Bagchi GD, Singh A, Khanuja SPS, Bansal RP, Singh SC, Kumar S. Wide spectrum antibacterial and antifungal activities in the seeds of some coprophilous plants of north Indian plains. J Ethnopharmacol 1999; 64: 69–77.
9. Nascimento GGF, Locatelli J, Freitas PC, Silva GL. Antibacterial activity of plant extracts and photochemical on antibiotic resistant bacteria. Braz J Microbiol 2000; 31: 247-256.
10. Ahmad L, Beg AZ. Antibacterial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. J Ethnopharm 2001; 74: 113-133.

11. Rajakaruna N, Harris CS, Towers GHN. Antimicrobial activity of plants collected from Serpentine outcrops in Sri Lanka. Pharmaceutical Biol 2002; 40(3): 235-244.
12. Shafi PM, Rosamma MK, Jamil K, Reddy PS. Antibacterial activity of *Syzygium cumini* and *Syzygium travancoricum* leaf essential oils. Fitoterapia 2002; 73(5): 414-416.
13. Chandrasekaran M, Venkatesalu V. Antibacterial and antifungal activity of *Syzygium jambolanum* seeds. J. Ethnopharm. 2004; 91: 105-108.
14. Ratnam KV, Raju RRV. In vitro antimicrobial screening of fruit extract of two *Syzygium* species (Myrtaceae). Adv Biol Res 2008; 2(1-2): 17-20.
15. Grover JK, Vats V, Rathi SS. Anti-hyperglycemic effect of *Eugenia jambolana* and *Tinospora cordifolia* in experimental diabetes and their effects on key metabolic enzymes involved in carbohydrate metabolism. J Ethnopharma 2000; 73(3): 461-470.
16. Muruganandan S, Srinivasan K, Chandra S, Tandan SK, Lal J, Raviprakash V. Antiinflammatory activity of *Syzygium cumini* bark. Fitotherapia 2001; 72: 369-375.
17. Houghton PJ, Raman A. Laboratory handbook for fractionation of natural extracts. Chapman and Hall, London. 1998.
18. Perez C, Paul M, Bazerque P. An Antibiotic assay by the agar well diffusion method. Acta Bio Med Exp 1990; 15: 113-115.
19. Ogbonnia SO, Enwuru NV, Onyemenem EU, Oyedele GA, Enwuru CA. Phytochemical evaluation and antibacterial profile of *Treculia africana* Decne bark extract on gastrointestinal bacterial pathogens. Afr J Biotech 2008; 7(10): 1385-1389.
20. Patel PR. Study of certain physiological and histo-architectural changes associated with the growth and ripening of some underutilized fruits. Ph. D. Thesis, Sardar Patel University Gujarat, India. 2009.
21. Allero AA, Afolayan AJ. Antimicrobial activity of *Solanum tomentosum*. Afr J Biotech 2006; 5: 369-372.
22. Parekh J, Chanda S. Antibacterial and phytochemical studies on twelve species of Indian medicinal plants. Afr J Biol Res 2007; 10: 175-181.
23. Shaikh MR, Baqir-maleka FA, Naqvi S. Partial purification and antibacterial studies of extracts from *Eugenia jambolana* Linn and *Vinca rosea* Linn. Pak J Sci Ind Res 1994; 37(6-7): 279-280.
24. Berenbaum M. Phototoxicity of plant secondary metabolites: Insect and mammalian perspectives. Arch Insect Biochem Physiol 1995; 29: 119–134.
25. Sagrawat H, Mann AS, Kharya MD. Pharmacological potential of Eugenia jambolana: A Review. Pharmacog Mag 2006; 6(2): 96-105.
26. Jamine R, Daisy P, Selvekumar BN. *In vitro* Efficacy of Flavonoids from *Eugenia jambolana* seeds against ESL-producing multidrug-resistant enteric bacteria. Res J Microbiol 2007; 2(4): 369–374.
27. Prasad, N. R., Viswanathan, S., Renuka Devi, J., Vijayashree N., Sweth, V. C., Archana Parathasarathy, N and Johanna R., 2008. Preliminary phytochemical screening and antimicrobial activity of *Samanea saman*. J Med Pl Res 2: 268-270.