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 was 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 ripened 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 tribes/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 fruit such as, it enriches the blood; strengthens teeth and gums; useful astringent in bilious diarrhoea; 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 antihypertensive,13,14 antidiabetic15 and anti-inflammatory16 activities. Thus, the plant is medically 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 using agar well diffusion method.18 All the bacterial cultures 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.18 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 colourless in case of dead bacterial population, using 2, 3, 5-triphenyl tetrazolium chloride test.19 The solutions containing
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 15 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 (15 mm). 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

<table>
<thead>
<tr>
<th>Extracts used</th>
<th>Stages of fruit growth and ripening</th>
<th>Zone of Inhibition (mm)</th>
<th>Gram +ve Bacteria</th>
<th>Gram -ve Bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>BC  BS  MS  SE  EC  KP  SP  ST</td>
<td>Gram +ve Bacteria</td>
<td>Gram -ve Bacteria</td>
</tr>
<tr>
<td>Diethyl ether</td>
<td>Young</td>
<td>10  9  3  6  7  1  -  7</td>
<td>Bacillus cereus</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Premature</td>
<td>13  5  2  4  -  2  -  8</td>
<td>Bacillus subtilis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mature</td>
<td>15  4  3  7  2  5  -  6</td>
<td>Escherichia coli</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Preripened</td>
<td>20  3  5  4  9  3  -  7</td>
<td>Staphylococcus epidermidis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ripened</td>
<td>13  4  6  4  6  2  -  12</td>
<td>Micrococcus luteus</td>
<td></td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>Young</td>
<td>4   1  -  4  1  2  -  3</td>
<td>Bacillus subtilis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Premature</td>
<td>2   1  -  -  -  5  2  -  2</td>
<td>Staphylococcus epidermidis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mature</td>
<td>5   2  -  2  3  -  2  -  2</td>
<td>Micrococcus luteus</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Preripened</td>
<td>2   4  5  2  5  -  4  5</td>
<td>Bacillus cereus</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ripened</td>
<td>4   3  3  4  4  -  5  5</td>
<td>Bacillus subtilis</td>
<td></td>
</tr>
<tr>
<td>Acetone</td>
<td>Young</td>
<td>2   5  1  1  3  3  5  3</td>
<td>Escherichia coli</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Premature</td>
<td>7   3  4  4  3  6  3  3</td>
<td>Staphylococcus epidermidis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mature</td>
<td>7   4  5  3  3  9  2  2</td>
<td>Micrococcus luteus</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Preripened</td>
<td>4   6  4  4  7  7  3  3</td>
<td>Bacillus cereus</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ripened</td>
<td>2   3  9  1  5  5  6  5</td>
<td>Bacillus subtilis</td>
<td></td>
</tr>
<tr>
<td>Methanol</td>
<td>Young</td>
<td>4   9  12 13 7  5  8  10</td>
<td>Klebsiella pneumonia</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Premature</td>
<td>8   8  11 11 7  6  11 8</td>
<td>Micrococcus luteus</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mature</td>
<td>4   7  12 7  6  3  9  9</td>
<td>Bacillus cereus</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Preripened</td>
<td>4   6  8  15 9  3  4  8</td>
<td>Bacillus subtilis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ripened</td>
<td>9   9  15 9  9  9  11 9</td>
<td>Escherichia coli</td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>Young</td>
<td>9   4  6  5  3  4  5  5</td>
<td>Staphylococcus epidermidis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Premature</td>
<td>9   6  4  7  4  4  8  4</td>
<td>Micrococcus luteus</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mature</td>
<td>5   5  7  8  3  4  8  5</td>
<td>Bacillus cereus</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Preripened</td>
<td>3   5  5  9  6  3  8  5</td>
<td>Bacillus subtilis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ripened</td>
<td>5   6  12 10 9  5  10 13</td>
<td>Salmonella typhi</td>
<td></td>
</tr>
</tbody>
</table>
Besides, the essential oil of Jamun leaves are credited
observed by
monoterpene aldehydes.24 Besides, the fruits have been reported to
antibacterial activity in methanol and ethyl-acetate extracts of
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.2 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. cumini against Gram-positive and Gram negative bacteria and
fungi.18 Bhuiyan et al. has obtained good antibacterial activity against
five gram positive and nine gram-negative bacterial strains.7 Besides, the
essential oil of Jamun leaves 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 to phytocomps such as
anthocyanins, alkaloids, canthoendric 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
2. Rao MR, Palada MC, Becker BN. Medicinal and aromatic plants
4. Warrier PK, Nambiwar VPK, Ramankutty C. Indian Medicinal
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
6. Zhang LL, Lin YM. Antioxidant tannins from Syzygium cumini
7. Bhuiyan MSA, Mia MY, Rashid MA. Antibacterial principles of
the seeds of Eugenia jambolana. Bangladesh J Bot 1996; 25(2):
239-241.
Wide spectrum antibacterial and antifungal activities in the
seeds of some coprophilous plants of north Indian plains. J
activity of plant extracts and photochemical on antibiotic
10. Ahmad L, Beg AZ. Antibacterial and phytochemical studies on
45 Indian medicinal plants against multi-drug resistant human


