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Research Article

Antimicrobial activities of seeds of *Diospyros blancoi*
and *Baccuarea ramiflora*.

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ABSTRACT

Diospyros blancoi and *Baccuarea ramiflora* are two common plants grown in Bangladesh for their fruits. Apart from the huge consumption of these fruits, different parts of these plants are widely used in the traditional medicine to treat different diseases. *D. blancoi* is used in diarrhea, dysentery, fever, itchy skin, cough and wounds whereas *B. ramiflora* is used in rheumatoid arthritis, cellulitis, abscesses and injury. To investigate the possible antimicrobial activity of the seeds of these two fruits, methanol extracts are prepared and tested against 10 pathogenic gram positive and gram negative bacteria by using disc diffusion method. Broth dilution assay was used to determine the minimum inhibitory concentration (MIC) of the extracts. The extract of *D. blancoi* showed significant antimicrobial activity against all of the tested bacteria whereas the antimicrobial activity shown by *B. ramiflora* is moderate. The MIC values for *D. blancoi* is lower for all of the bacteria compared to the MIC values for *B. ramiflora*. The results of the present study indicate that the seed extracts of *D. blancoi* has significant antimicrobial activity which justifies its use in traditional treatment of various infectious conditions.

Keywords: *Diospyros blancoi*, *Baccuarea ramiflora*, medicinal plant, antimicrobial activity.

INTRODUCTION

Diospyros blancoi A. DC (Ebenaceae) locally known as 'bilati gab', is a popular fruit in Bangladesh. The fruit of this plant is commonly known as mabolo and wood is known as kamagong¹. This plant is an endemic Philippine tree though it is cultivated widely in India and China for its huge consumption¹. The bark of this plant is traditionally used in the treatment of diarrhea, dysentery, fever and cough¹. The fruit is used in wounds and aphthous stomatitis while the leaves and barks are used in itchy skin¹. Previous studies on chemical composition of the genus of *diospyros* have reported the presence of triterpenes: lupeol, maslinic acid, betulin, betulinic acid, lupeol acetate, ursolic acid, oleanolic acid, taraxerol² and lanostane³. Aromatic compounds such as plumbagin, diospyrin, isodiospyrin, diospyrol, astragalol, kaempferol glucoside, isoquercetin and quercetin glucoside were also isolated from this genus. Roots of this plant contain lupeol, habibone, and diospyrin 80-hydroxydiospyrin⁴. Further biological studies on these isolated chemical compounds exerted various pharmacological activities³. Isoarborinol methyl

ether, a mixture of -amyryl palmitate, -amyryl palmitoleate, -amyryl palmitate and -amyryl palmitoleate and squalene are elucidated from the leaves of *D. blancoi* which further exhibited significant antimicrobial, analgesic¹, antioxidant¹, antidiarrhoeal⁵ and anti-inflammatory¹ activities. Studies on fruits of *D. blancoi* revealed the presence of 96 volatile compounds among them esters, particularly benzyl butyrate, butyl butyrate and (E)-cinnamyl butyrate are common⁶. The leaf extract of *D. blancoi* exhibited anti-inflammatory activity in airway inflammation and allergic bronchial asthma⁷. *Baccuarea ramiflora* Lour. (Euphorbiaceae) locally known as 'Latkan' in Bangladesh, is a tall evergreen tree native to Southeast Asia region and grows widely in Bangladesh, India, Thailand, Burma, Vietnam, Laos, Cambodia, Malaysia and China^{8,9}. The tree usually reaches a height of about 5-10 m. Fruit is yellowish and velvety, 2-3 cm in diameter with leathery pericarp, three seeded arillus embedded in pinkish white pulp¹⁰. Traditionally the plant is used in rheumatoid arthritis, cellulitis, abscesses and injury

from fall as an anti-inflammatory and anodyne^{11, 12}. The fruit juice is mainly used for the treatment of constipation⁸. They are also stewed or made into wines in Malaysia and India. It has been further established that phenolics and flavonoid constituents present in wine have therapeutic potential as antioxidant and anti-inflammatory agents¹³⁻¹⁵. Because of the high content of vitamin C, protein and iron *B. ramiflora* fruit finds its importance as a novel food additive¹¹. Studies on the fruit pulp have revealed the presence of total phenols, flavonoids, flavonols and proanthocyanidins as well as antioxidant property⁸. Previous studies on the chemical constituents have reported two phenols, 6'-O-vanilloylisotachioside and 6'-O-vanilloyltachioside from the leaves¹⁶, 4-O-(6-O-vanilloyl)-D-glucopyranosyl tachioside D, 6-O-vanilloyl picraquassioside D and 6-O-vanilloylcariside B5 from the stems¹⁷, one new nor-picrotoxane sesquiterpene glycoside; ramifloside and two picrotoxane sesquiterpenes sapidolide A and picrotoxinaesin from the berries¹⁸ and a series of volatile components from the plant of *B. ramiflora*¹⁶. Biological studies of various compounds isolated earlier from *B. ramiflora* have exhibited significant antioxidant⁸, antifungal¹⁸ and anti-inflammatory¹⁵ properties.

D. blancoi and *B. ramiflora* both are popular fruits of Bangladesh. Besides their use as fruit, various parts of these two plants are also used by the people from ancient time as a traditional medicinal plant for the ailment of various health conditions. To the best of our knowledge, no antimicrobial studies have been performed so far on the seeds of *D. blancoi* and *B. ramiflora*. Keeping this in mind the present work deals with the screening of antimicrobial activity of the seeds of *D. blancoi* and *B. ramiflora*.

MATERIALS AND METHODS

Plant materials and Extract Preparation

Mature fruits of *D. Blancoi* was collected from Manikgonj district and *B. ramiflora* was collected from Narsingdi district of Bangladesh in 2013. The collected samples were authenticated by the experts of National Herbarium, Mirpur, Dhaka, Bangladesh (Accession No. 38439 and 38438 respectively) where a voucher specimen has been deposited for further reference. The peels and pulps of the fruits have been separated from the seeds and the seeds were then dried and grounded. 300 g of each of the dried powders were taken in two different beakers and 450 ml of methanol was added in both of the beakers and kept for seven days with occasional stirring. The filtrates were then collected and dried to get the extracts¹⁹.

Microorganisms

Pure cultures of four gram positive bacteria such as *Staphylococcus aureus*, *Sarcina lutea*, *Bacillus subtilis* and *Clostridium spp.* and six gram negative bacteria such as *Salmonella typhimurium*, *Escherichia Coli*, *Shigella boydii*, *Pseudomonas spp.*, *Vibrio cholerae* and *Klebsiella pneumoniae* were collected from the Microbiology laboratory of Department of Pharmacy, Primeasia University, Dhaka, Bangladesh (106 CFU/ml).

Screening of antimicrobial activity

Antimicrobial activities of methanol extracts of the seeds of *Diospyros blancoi* (SDB) and *Bacchara ramiflora* (SBR) were evaluated by disc diffusion method²⁰. Azithromycin (30 µg/disc) (Oxoid, UK) were used as positive control and the extract solutions of SDB and SBR were prepared at a concentration of 300 µg/ml. 6 mm filter paper discs were impregnated by using the extracts and placed onto the nutrient agar media which were then inoculated with the test bacteria and incubated at 37°C for 24 h. Blank discs (impregnated with solvents followed by evaporation) were used as negative control to investigate the involvement of the solvent for the given activity (if any). After incubation the culture plates were examined and the zones of inhibition were measured in mm scale. Triplicate experiments were performed for each organism.

Determination of minimum inhibitory concentration (MIC)

Broth dilution method was used to determine the MIC of the extracts²¹. Briefly, extract solution at a concentration of 200 mg/ml were taken from which 1.0 ml of the reconstituted solution was added to another test tube containing 1 ml of sterile broth so as to obtain a concentration of 100mg/ml. In this way 10 times dilution was performed and the 11th test tube did not contain any extract, but a solution of pure solvent served as negative control. Then 1 ml of an 18 h old culture of each of the organisms earlier adjusted at 106 CFU/ml was put into each tube and thoroughly mixed. The tubes were incubated at 37°C for 24 h and observed for growth of bacteria in form of turbidity. The test tube with the lowest dilution with no detectable growth by visual inspection was considered the MIC.

RESULTS

Antimicrobial Activity

The antimicrobial activities of the methanol extracts of SDB and SBR are shown in Table 1. The zones of inhibition given by the standard antibiotic disc

Azithromycin (30 µg/disc) were compared with the zones of inhibition given by the extracts of SDB and SBR. This study revealed that SDB extract showed significant zones of inhibition against all of the tested bacteria whereas the extract of SBR did not give any zone of inhibition for *Sarcina lutea* and *Bacillus subtilis*. The order of zone of inhibition for SDB is *Staphylococcus aureus* (20.33 mm) > *Vibrio cholerae* (19.67 mm) > *Klebsiella pneumoniae* (18.67 mm) > *Clostridium spp.* (17.67 mm) > *Sarcina lutea* (16.33 mm) > *Salmonella typhimurium* (15.33 mm) > *Bacillus subtilis*, *Shigella boydii* (14.67 mm) > *Escherichia Coli* (13.33 mm) > *Pseudomonas spp.* (12.33 mm). The extract of SBR gives lower inhibition zones against the tested bacteria which ranges from *Klebsiella pneumoniae* (09.33 mm) to *Shigella boydii* (13.67 mm) and in between the range.

Standard antibiotic Azithromycin produced significant zones of inhibition against the tested microorganisms and the negative control does not give any zone of inhibition for any of the tested bacteria which implies that the solvent does not involve in the antimicrobial activities given by the extracts.

Minimum inhibitory Concentration (MIC)

Minimum inhibitory concentrations for SDB and SBR are shown in Table 2. MIC values of SDB extract were found between 1.6-6.3 mg/ml whereas MIC values of SBR extract ranged between 3.2-12.5 mg/ml. From the result it is evident that *Staphylococcus aureus*, *Sarcina lutea*, *Salmonella typhimurium* and *Escherichia Coli* were inhibited with the concentration of 1.6 mg/ml and *Pseudomonas spp.* required the concentration of 6.3 mg/ml in case of the extract of SDB. The results of the MIC values for the extract of SBR showed that increased concentrations are required to inhibit the growth of the tested organisms and the highest concentration of 12.5 mg/ml was required to inhibit the growth of *Salmonella typhimurium* and the lowest concentration 3.2 mg/ml is required to inhibit the growth of *Staphylococcus aureus*, *Sarcina lutea* and *Shigella boydii*.

DISCUSSION

From the distant past it has been recognized that medicinal plants and their extracts have antiseptic properties while attempts to characterize these properties in the laboratory started from the early 1900s²². Investigations of plants possessing antimicrobial activities and their possible mode of action and potential uses in the traditional practices intended to protect human, livestock and food from diseases, pests and spoilage have gained major

attention now a days²². In developing countries infectious diseases impose a big threat to public health probably due to the unavailability and high cost of medicines²³. A good proportion of world's population, particularly from the developing countries rely on plants and plant derived antimicrobial agents for the treatment of infectious and non infectious diseases²⁴. In the present study methanol extracts of seeds of *D. blancoi* and *B. ramiflora* were investigated for possible antimicrobial properties based on their traditional use against 10 pathogenic gram positive and gram negative bacteria. Between the two extracts it is evident from the result that the extract of SDB has more antimicrobial potential than the extract of SBR. SDB showed most significant inhibition zone against *Staphylococcus aureus* (20.33 mm) while the lowest zone of inhibition has been found against *Pseudomonas spp.* (12.33 mm). The data obtained from this study showed that the extract of SBR has mild antimicrobial potential though the maximum zone of inhibition it showed against *Shigella boydii* (13.67 mm) and for the other bacteria the zone of inhibition are lower than this value. To measure the effectiveness of antimicrobial agents which may be predictive of therapeutic outcome, determination of the MIC is an effective way. MIC and antimicrobial activity can be correlated in a manner that they shares inversely proportional relationship, as agents with lower activity usually gives higher MIC, while a highly reactive agent gives lower MICs against a particular organism. In the present study the MIC given by the extracts and their antimicrobial results also follows similar pattern. As the extract of SDB gives strong antimicrobial potential, it also requires lower concentration to inhibit the growth of the organisms while the extract of SBR requires a higher concentration.

Previous reports on *B. ramiflora* revealed that the fruit contains flavonoids, phenols, proanthocyanidins and picrotoxane sesquiterpene which might be responsible for giving the antimicrobial property of the plant^{8,18}. Moreover the picrotoxane sesquiterpene also possess antifungal activity, this is also supportive to the antimicrobial property of the plant. Previous phytochemical reports revealed the presence of flavonoid, sterols, saponnins, terpenes, gum, sugar, tannins, alkaloids and phenolic acids in *D. blancoi*⁵. Studies on the correlation between the presences of these plant secondary metabolites and their antimicrobial potential has already been established⁵. In the present study as the extract showed significant antimicrobial activity, it is certain that there is a correlation between the presence of the secondary

metabolites and the antimicrobial activity exhibited by the extract of *D. blancoi*.

CONCLUSION

The findings of the present study affirmed the antimicrobial potential of the seeds of *D. blancoi* and *B. ramiflora* which can be correlated with their traditional uses in various infectious conditions. The findings of this study can be used further to isolate

the possible chemical constituents responsible for this given activity in various animal models.

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Table 1
Antimicrobial activities of the extracts of SDB and SBR using Disc Diffusion Method.

Test organisms	Diameter of inhibition Zone in mm scale		
	Azithromycin (30 µg/disc)	SDB (300 µg/disc)	SBR (300 µg/disc)
<i>Staphylococcus aureus</i>	40	20.33 ± 0.94	10.33 ± 0.47
<i>Sarcina lutea</i>	37	16.33 ± 1.25	--
<i>Bacillus subtilis</i>	27	14.67 ± 1.25	--
<i>Clostridium spp.</i>	26	17.67 ± 0.47	09.67 ± 1.70
<i>Salmonella typhimurium</i>	34	15.33 ± 0.47	12.67 ± 1.25
<i>Escherichia Coli</i>	40	13.33 ± 0.47	10.67 ± 0.94
<i>Shigella boydii</i>	30	14.67 ± 1.25	13.67 ± 0.94
<i>Pseudomonas spp.</i>	28	12.33 ± 0.94	10.33 ± 1.25
<i>Vibrio cholerae</i>	36	19.67 ± 1.25	11.67 ± 0.94
<i>Klebsiella pneumoniae</i>	42	18.67 ± 1.70	09.33 ± 2.05

*Data are expressed as mean ± SD of triplicate experiments.

Table 2
Minimum inhibitory concentration (MIC) of the extracts of SDB and SBR.

Test organisms	MIC of SDB in mg/ml	MIC of SBR in mg/ml
<i>Staphylococcus aureus</i>	1.6	3.2
<i>Sarcina lutea</i>	1.6	3.2
<i>Bacillus subtilis</i>	3.2	6.3
<i>Clostridium spp.</i>	3.2	6.3
<i>Salmonella typhimurium</i>	1.6	12.5
<i>Escherichia Coli</i>	1.6	6.3
<i>Shigella boydii</i>	3.2	3.2
<i>Pseudomonas spp.</i>	6.3	6.3
<i>Vibrio cholerae</i>	3.2	12.5
<i>Klebsiella pneumoniae</i>	3.2	6.3

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