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Bridelia retusa (L.) Spreng. Fruits: Antimicrobial Efficiency and their Phytochemical Constituents

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Abstract

Antimicrobial analysis of hexane, chloroform, methanol and aqueous extracts of *Bridelia retusa* fruits was performed by agar well method and minimum inhibitory concentration was determined by serial two-fold dilution method. Seven human pathogenic bacteria species including Gram positive and Gram negative bacteria and three fungal species were used in the study and the results indicated that the Gram positive bacteria and fungi were more sensitive than the Gram negative bacteria, to both solvent and aqueous *Bridelia retusa* fruit extracts. Moreover, *Enterococcus faecalis* was found as the most sensitive bacteria, whereas *Proteus vulgaris* and *Aspergillu niger* were the most resistant to the tested extracts. Phytochemical analysis of fruits revealed the presence of secondary metabolites like alkaloid, saponins and terpenoids, which have been implicated in antimicrobial activities. Hence, it would be recommended to explore the maximum potential of *Bridelia retusa* in the medicinal and pharmaceutical field and investigation are endorsed for further application useful in phytomedicine.

Keywords: antimicrobial activity, *Bridelia retusa*, fruit extracts, phytochemical

Introduction

Bridelia retusa Spreng. (family Euphorbiaceae) is a small to moderate sized deciduous tree, found in India, Bangladesh, Nepal, Sri Lanka, Southern China, Indochina and Sumatra. Traditionally, it is valuable as astringent, used in rheumatism problems, urinary infections; the plant promote antifertiliy and wound healing. Stem bark is used to treat dysentery, diarrhea and diabetes. Leaves and fruits are used as antifungal and for stomach ache (Mishra and Sahu, 1984; Nadkarni and Nadkarni, 2000; Jayasinghe *et al*., 2003). These different pharmacological properties are due to the presence of different chemical constituents as isoflavone (Adhavet, 1998), decanoic acie, stigmasterol, dehydrostigmasterol, β-sitosterol, tannins and triterpenes. Fruit pulp contains gallic acid, ellagic acid and β-sitosterol (Malhotra and Moorthy, 1973). It is well known also for the presence of tannins. It is reported to be used traditionally in snake bites, wounds and tonics for veterinary purposes (Joshi *et al*., 1980). Phenolics, including tannins, are the natural products present in abundant amount and possess various biological properties related to anti inflammatory effects (Mehare and Hatapkki, 2003), wound healing activity (Bagad, 2007), antioxidant (Tatiya *et al*., 2011), antimicrobial activity of stem bark (Tatiya *et al*., 2011; Kurdekar *et al*., 2012) or leaves (Khan and Khan, 2013). Various parts of the plants were reported for antimicrobial activity, but there are no reports on antimicrobial activity of fruits, hence the present investigation was carried out. Thus the antimicrobial activities of both solvent and aqueous extracts of *B. retusa* fruits were analyzed.

Material and Methods

Collection, identification and extraction

Bridelia retusa (L.) Spreng. fruits were collected from Kambalakonda forest area, Visakhapatnam, Andhra Pradesh, India. The collected fruits were identified by Prof. M. Venkaiah, Department of Botany, Andhra University, Visakhapatnam, India. The collected fruits were dried in the shadow until completely dried. Then the dried fruits were powdered in the mixture grinder and packed in Soxhlet apparatus. Sequential extraction was done using hexane, chloroform, followed by methanol. The filtrates were concentrated by removing the solvents under reduced pressure, at 40 °C, using a rotary evaporator. The concentrated crude extracts were labeled and stored at 4 °C.

Simultaneously, the aqueous extract of the fruits was prepared by adding boiled water to the powdered fruits in a beaker on water bath, with occasional stirring for 4 hrs. The aqueous extract was then filtered and reduced under pressure.

Bacterial and fungal strains used

The strains used within the experiment were procured from the Microbial Type Culture and Collection (MTCC), Chandigarh, India. Seven bacterial strains namely *Bacillus subtilis* MTCC B2274, *Enterococcus faecalis* MTCC B3159, *Micrococcus luteus* MTCC B1538, *Staphylococcus aureus* MTCC B3160, *Streptococcus pneumoniae* MTCC B2672, *Escherichia coli* MTCC B1560, *Klebsiella pneumoniae* MTCC B4030, *Pseudomonas aeruginosa* MTCC B2297, *Proteus vulgaris* MTCC B7299, and three fungal strains such as *Aspergillus niger*

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Table 1. The antimicrobial activity of *Bridelia retusa* fruit extracts against various pathogens

Organisms	Zone of inhibition (mm)[a]												S	D
	Hexane extract			Chloroform extract			Methanol extract			Aqueous extract				
	25 mg/ml	50 mg/ml	100 mg/ml	25 mg/ml	50 mg/ml	100 mg/ml	25 mg/ml	50 mg/ml	100 mg/ml	25 mg/ml	50 mg/ml	100 mg/ml		
B. subtilis	12±0.4	13±0.4	14±0.4	16±0.1	18±0.9	21±0.4	15±0.1	17±0.9	19±0.9	16±0.4	18±0.4	21±0.4	18^{T}	–
E. faecalis	18±0.2	20±0.4	21±0.4	19±0.7	22±0.5	25±0.2	23±0.5	26±0.9	28±0.5	16±0.4	20±0.4	23±0.4	21^{T}	–
M. letus	10±0.1	13±0.1	13±0.9	13±0.2	14±0.1	18±0.5	13±0.4	16±0.1	18±0.4	10±0.1	13±0.5	14±0.1	24^{T}	–
S. aureus	12±0.9	15±0.3	17±0.9	20±0.4	21±0.1	23±0.2	20±0.4	22±0.7	24±0.2	15±0.9	18±0.9	21±0.9	24^{T}	–
S. pneumoniae	16±0.4	17±0.4	20±0.2	20±0.9	23±0.7	24±0.9	21±0.2	25±0.4	27±0.5	20±0.9	20±0.4	23±0.9	22^{T}	–
E. coli	10±0.4	10±0.4	10±0.9	10±0.6	12±0.4	12±0.4	13±0.1	15±0.5	17±0.2	12±0.4	12±0.1	13±0.2	22^{T}	–
K. pneumoniae	10±0.4	10±0.4	12±0.4	12±0.4	14±0.4	16±0.4	13±0.2	16±0.1	19±0.1	13±0.4	14±0.4	14±0.4	24^{T}	–
P. aeruginosa	10±0.4	11±0.4	13±0.4	10±0.4	12±0.4	14±0.4	11±0.5	14±0.6	16±0.2	10±0.7	11±0.6	12±0.1	25^{T}	–
P. vulgaris	-	-	-	-	-	-	-	-	-	-	-	-	22^{T}	–
A. niger	-	-	-	-	-	-	-	-	-	-	-	-	18^{N}	–
C. albicans	13±0.4	14±0.4	15±0.4	22±0.4	24±0.4	26±0.5	22±0.2	26±0.5	29±0.2	16±0.4	19±0.4	23±0.4	23^{N}	–
S. cerevisiae	14±0.7	16±0.1	18±0.1	17±0.9	19±0.2	21±0.2	19±0.1	21±0.9	23±0.2	16±0.4	17±0.4	21±0.4	20^{N}	–

a: each value is the mean of three replicates with standard deviation; *P* value is <0.001, extremely significant when compared with standard; S: standard antibiotics; T- tetracycline; N- nystatin; D: DMSO; –: no activity

Table 2. MIC values of *Bridelia retusa* fruit extracts against tested microorganisms

Microorganism	Hexane extract (µg/ml)	$ChCl_3$ extract (µg/ml)	Methanol extract (µg/ml)	Aqueous extract (µg/ml)
B. subtilis	500	500	125	500
E. faecalis	62.5	62.5	31.2	31.2
M. letus	1000	250	125	1000
S. aureus	500	125	62.5	125
S. pneumoniae	250	125	31.2	62.5
E. coli	>1,000	1,000	500	500
K. pneumoniae	>1,000	500	250	500
P. aeruginosa	>1,000	1,000	500	1000
P. vulgaris	>1,000	>1,000	>1,000	>1,000
A. niger	>1,000	>1,000	>1,000	>1,000
C. albicans	1,000	62.5	31.2	62.5
S. cerevisiae	250	62.5	125	250

MTCC F4325, *Candida albicans* MTCC F7315 and *Saccharomyces cerevisiae* MTCC F2567 were used for testing the antimicrobial effect of *Bridelia retusa* (L.) Spreng. fruit extracts.

Antimicrobial efficiency

The lyophilized culture was sub-cultured and concentration of working stock culture was assessed as 10^{-6} CFU/ml. Specified quantity of nutrient agar was prepared and plated in aseptic conditions. The agar well diffusion technique was performed for antimicrobial susceptibility test for crude extracts and dimethyl sulfoxide (DMSO), whereas agar disc diffusion method was followed for antimicrobial susceptibility test for standard antibiotic discs. The extracts were dissolved in DMSO to get the known concentrations of 25 mg/ml, 50 mg/ml and 100 mg/ml respectively. After 24 h of incubation at 37 °C, the zone of inhibition was measured using an antibiotic zone reader scale (HiAntibiotic ZoneScale-c) and tabulated. For the antifungal activity, the same method as for bacteria, of nutrient agar respectively, was adopted, whereas Saboraud dextrose agar was used. The inoculated medium was incubated at 25 °C for two days for the *C. albicans*, and *S. cerevisiae* and three days for *A. niger* (Aniel Kumar *et al*., 2014). About 500 µg of nystatin was dissolved in 1 ml of sterile de-ionized water. About 10 µl of 0.5 mg/ml nystatin (equivalent to 5 µg dose) and 10 µl of DMSO was pipetted into wells. For bacteria, multidrug antibiotic disc was used (Axiom Laboratories Ltd., India). The experiments were conducted in triplicates each and diameter of the inhibition zone surrounding each well was recorded and tabulated.

The extracts that exhibited inhibition zones were subjected to minimum inhibition concentration (MIC) assay by using serial two-fold dilution method (Aniel Kumar *et al*., 2010). A quantity of 0.6 g of each extract was dissolved in 300 ml sterile nutrient broth which yielded initial concentration of 2,000 µg/ml. Subsequently, serial dilution was made from the stock to obtain 1,000; 500; 250; 125; 62.5; 31.2 µg/ml concentrations. One ml of standardized inoculums of each test organism was introduced into each extract, nutrient broth mixture and then incubated at 37 °C. The lowest concentration inhibiting growth of microorganism was regarded as the MIC of the extracts. For the fungi, the inoculated medium was incubated at 25 °C for two (*C. albicans*, *S. cerevisiae*) to three (*A. niger*) days.

Statistical analysis

Each experimental data from triplicates was subjected to one way ANOVA using Minitab version 15. A significant level of $p < 0.01$ was used for all statistical analyses.

Results and Discussion

The antimicrobial activity of fruit extracts of *B. retusa* was assayed by agar well diffusion method against seven bacterial strains including Gram positive *B. subtilis, E. faecalis, M. luteus, S. aureus, S. pneumoniae*, Gram negative *E. coli, K. pneumoniae, P. aeruginosa, P. vulgaris*, fungi *A. niger, C. albicans* and *S. cerevisiae*. Table 1 shows the microbial growth inhibition zones

Table 3. Phytochemical constituents of *Bridelia retusa* fruits

Phytochemicals	Hexane extract	$ChCl_3$ extract	Methanol extract	Aqueous extract
Alkaloids	+	+	+	+
Aminoacids	+	+	+	+
Anthraquinone	-	-	+	-
Carbohydrates	+	+	+	+
Cardiac glycosides	-	+	+	-
Flavonoids	+	+	+	+
Glycosides	+	+	+	+
Phenols	-	+	+	-
Saponins	-	+	+	-
Steroids	+	+	+	+
Tanins	-	-	+	+
Terpenoids	+	+	+	-
Volatile compounds	+	+	+	+

of both solvent and aqueous extracts of *B. retusa* fruits. All extracts found to be effective against all tested bacteria and fungi except that of *P. vulgaris* and *A. niger*. Chloroform, methanol and aqueous extracts exhibited the antimicrobial activity with the maximum zone of inhibition against *C. albicans*, while hexane extract was most effective against *E. faecalis*; aqueous extract exhibited the maximum zone of inhibition against *E. faecalis* and *S. pneumoniae* along with *C. albicans*.

E. faecalis exhibited inhibition zones similar or larger than standard antibiotic tetracycline, while *B. subtilis, S. pneumoniae, C. albicans* gave better results for chloroform, methanol and aqueous extracts; *S. aureus* had a larger inhibition zone in the case of methanol extract. Methanol and aqueous extracts also showed high inhibition zones against fungal strains *C. albicans* and *S. cerevisiae*. Although the Gram negative bacteria were sensitive for all extracts, did not show a broad spectrum of antimicrobial activity. The negative control of DMSO had no effect on the microbial growth of all tested bacteria and fungi. Hence, the absence of inhibition zones confirmed that DMSO could not act as antimicrobial agent.

The effect of different solvents such as water, ethanol (50%), methanol (50%) and acetone (70%) of *B. retusa* stem bark exhibited antimicrobial activity against Gram positive bacteria *B. subtilis, S. aureus* and Gram negative bacteria *E. coli* and fungi *C. albicans* (Tatiya *et al*., 2011), whereas in the present study *B. retusa* fruits extracts had also shown high antimicrobial activity against Gram positive bacteria *B. subtilis, E. faecalis, S. pneumoniae* and fungi *C. albicans*.

Table 2 shows the MIC values that were exhibited by solvent and aqueous extracts of *B. retusa* fruits. Methanol extract had low MIC values against *E. faecalis, S. pneumonia* and *C. albicans*, whereas aqueous extract was effective against *E. faecalis* at a concentration of 31.2 µg/ml concentration. The current findings are in agreement the results obtained with various extracts of *B. retusa* stem bark which have shown the strongest MIC values against fungi *C. albicans* and lowest effect against Gram positive bacteria *B. subtilis* (Tatiya *et al*., 2011).

Phytochemical analysis (Table 3) revealed that *B. retusa* fruits posses alkaloids, aminoacids, anthraquinone, carbohydrates, cardiac glycosides, flavonoids, glycosides, phenols, saponins, steroids, tannins, terpenoids and volatile compounds. Methanol extract exhibited positive results for all tested phytochemicals, whereas the observed antimicrobial activity may be due to the presence of some metabolites like alkaloid, saponins and terpenoids, which are implicated in various biological activities (Thomas *et al*., 2013). The presence of these metabolites suggests great potential for the plant as a source of useful phytomedicines (Kunle *et al*., 2003).

Conclusions

It may be concluded that the results of the present study support the folkloric usage of the *B. retusa* as a medicine. The results indicated that the Gram positive bacteria and fungi were more sensitive than the Gram negative bacteria, to both solvent and aqueous *Bridelia retusa* fruit extracts. Phytochemical analysis revealed that *B. retusa* fruits posses alkaloids, aminoacids, anthraquinone, carbohydrates, cardiac glycosides, flavonoids, glycosides, phenols, saponins, steroids, tannins, terpenoids and volatile compounds; antimicrobial activity may be due to the presence of some metabolites like alkaloid, saponins and terpenoids, which are implicated in various biological activities. Hence, it is necessary to explore the maximum potential of the plant in medicinal field and pharmaceutical sciences for further application. Further studies are required about the appropriate characterization of the compounds present in the *B. retusa* fruits.

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