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 Aspergillus 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 antifebrility 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 Mohr, 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 boiling 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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The lyophilized culture was sub-cultured and concentration of working stock culture was assessed as 10<sup>5</sup> 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 working stock culture was assessed as 10<sup>8</sup> CFU/ml.

### Antimicrobial efficiency

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### 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 <i>B. retusa</i> was assayed by agar well diffusion method against seven bacterial strains including Gram positive <i>B. subtilis, E. facalis, M. luteus, S. aureus, S. pneumoniae</i>, Gram negative <i>E. coli, K. pneumoniae, P. aeruginosa</i>, <i>P. vulgaris</i>, fungi <i>A. niger, C. albicans</i> and <i>S. cerevisiae</i>. Table 1 shows the microbial growth inhibition zones.
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. Hence, the absence of inhibition zones of DMSO had no effect on the microbial growth.

**Table 3. Phytochemical constituents of *Bridelia retusa* fruits**

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Hexane extract</th>
<th>CHCl3 extract</th>
<th>Methanol extract</th>
<th>Aqueous extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Aminocids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Anthraquinone</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Cardiac glycosides</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Phenols</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Saponins</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Steroids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Tannins</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Terpenoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Volatile compounds</td>
<td>+</td>
<td>+</td>
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</table>

**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, aminocids, 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 (Thomas *et al.*, 2013). The presence of these metabolites suggests great potential for the plant as a source of useful phytotherapeutics (Kunle *et al.*, 2003).

**References**


