Phytochemical studies and antibacterial activity of Barleria acuminata Nees (Acanthaceae)

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Abstract
The study was aimed to analyse preliminary screening of phytochemicals and evaluation the antibacterial activity of the leaves extracts of Barleria acuminata. Acetone, aqueous, dimethyl ether, chloroform and ethanol extracts of dried and ground plant materials were prepared using Soxhlet apparatus. Qualitative phytochemical screening of the extracts showed positive for alkaloids, flavonoids, tannins, steroids, phenols, terpenoids and saponin. Antibacterial activities of the extracts were evaluated by agar well diffusion method. The extracts exhibited significant effects against more bacterial pathogens and Gram negative bacteria were highly inhibited than Gram positive bacteria. The ethanol extracts of leaf showed more activity followed by chloroform extract. This study supports the traditional use of B.acuminata for the treatment of bacterial infectious diseases and might be helpful for further investigation of the plants to assess their chemical prospective in future research.

Keywords: phytochemical, antibacterial, Barleria acuminata, plant extracts

Introduction
India is endowed with a wealth of medicinal plants, which have been a valuable source of natural products for maintaining human health. Plants are the richest resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs [1]. Medicinal plants are widely used for the treatment of human diseases all over the world because they contain components with therapeutic value [2]. Since immemorial times, nature has been a source of these medicinal agents as these secondary metabolites (especially flavonoids) are synthesized by plants in response to microbial infection [3]. According to World Health Organisation (WHO) more than 80% of the world’s population relies on traditional medicine for their primary healthcare needs. Natural products play an important role in drug development in the pharmaceutical industry [4]. There are many reports on the use of medicinal plants in traditionally used by either tribal people or indigenous population [5]. The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization. Research on the biological activities of plants during the past two centuries has yielded numerous compounds for the development of modern drugs [6]. The medicinal value of plants lies in some chemical substance that produces a definite physiological action on the human body. The most important of these bioactive compounds are alkaloids, saponins, flavonoids, tannins and phenolic compounds [7]. Barleria acuminata is commonly known as Vellaikurunji. It is used as one of the most important drugs in traditional system of medicine. Acanthaceae [8]. The leaves are dark green on the upper surface and pale green on the lower surface. They are elliptic to narrowly ovoate. The flowers are about 5 cm long, funnel-shaped in violet, pink, or white color. Leaves to 4-5 x 2.5-3 cm, ovate-ovicular, apex acute, apiculate, base rounded, tomentose; petiole to 3.5 cm, pubescent [9]. The present study deals with the preliminary phytochemical screening and invito antibacterial activity of the plant extracts of Barleria acuminata.

Materials and Methods
Plant sample
Barleria acuminata leaves were collected and washed in running tap water to remove dust particles, shade dried at room temperature and ground into fine powder using electric chopper. About 30g of coarsely powdered leaves were successively extracted using Soxhlet apparatus with different solvents. The solvents used were Acetone, Chloroform, Dimethyl ether, Ethanol and Distilled water. The extracts were concentrated by gentle heating and stored for future use.
Phytochemical screening
Phytochemical screening tests were performed by standard protocols for detecting the presence of various classes of phytochemicals, includes alkaloids, vitamin C, flavonoids, tannins, steroids, phenols, phlobatannins, terpenoids, glycosides and Saponins.[10]

Antibacterial Assay
Antibacterial activity of the plant extracts were determined by agar well diffusion method[11] against five Gram positive bacteria viz. Bacillus cereus, bacillus subtilis, Enterococcus faecalis, Staphylococcus aureus and Staphylococcus epidermidis, and five Gram negative bacteria viz. Escherichia coli, Klebsiella pneumonia, Proteus mirabilis, Salmonella typhi and Shigella dysentriae. Briefly, fresh bacterial cultures of 0.1 ml having 108 colony forming unit were spread on Muller Hinton Agar plate using sterile cotton swab. The wells were punched off into agar medium with sterile well puncture and each well was filled with 50 μl of plant extract using micro pipette in aseptic condition. The plates were then kept in a refrigerator to allow pre-diffusion of the extract for 30 min and further incubated at 37 °C for 24 h. Antibacterial activity was evaluated by measuring the zone of inhibition with the help of Antibiotic Zone Scale-C.

Results
Phytochemical screening
The acetone extract of the plant showed positive for alkaloids, flavonoids, tannin, steroids and terpenoids; chloroform extract for flavonoids, tannin, steroids, phenols and terpenoids; dimethyl ether extract for flavonoids and steroids; ethanol extract for alkaloids, flavonoids, tannin, steroids, phenol and terpenoids; and water extract showed positive for alkaloids, steroids and saponin. Overall, the plant extracts showed positive for alkaloids, flavonoids, tannins, steroids, phenols, terpenoids and saponin (Table 1).

<table>
<thead>
<tr>
<th>Phytochemical Constituents</th>
<th>Presence (+) or Absence (-) in different extracts</th>
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<tbody>
<tr>
<td></td>
<td>Acetone</td>
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<tr>
<td>Alkaloids</td>
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<tr>
<td>Vitamin C</td>
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<td>Flavonoids</td>
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<td>Tannins</td>
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<td>Steroids</td>
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<td>Phenols</td>
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<td>Phlobatannins</td>
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<td>Terpenoids</td>
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<td>Glycosides</td>
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<td>Saponins</td>
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Antibacterial assay
Antibacterial activities of the extracts were evaluated by a zone of inhibition and the values are measured in mm. Gram positive bacteria were highly inhibited by ethanol extract followed by chloroform. Gram negative bacteria were highly inhibited chloroform followed by ethanol. The inhibition ranges were found to be 8 to 14 mm. Among the groups, Gram negative bacteria were highly inhibited by the plant extract of B. acuminata (Fig. 1).

Discussion
The preliminary phytochemical investigation of the medicinal plant B. acuminata leaf extracts revealed the presence of major secondary metabolites such as alkaloids, flavonoids, tannins, steroids, phenols, terpenoids and saponin. Among five solvents used ethanol and chloroform shows greatest positive results in phytochemical screening. The entire extracts were tested for antibacterial activity against pathogenic bacteria. Antibacterial activities of the extracts were ranged from 8 to 14 mm and Gram negative bacteria were highly inhibited than Gram positive bacteria. Also, among the five solvents used, ethanol extract affected more organisms followed by chloroform extract. Highest zone of inhibition (14 mm) was observed against S. dysentriae by chloroform extract of B. acuminata.
The phytochemical constituents of the plant products serve as a defense mechanism [11]. These metabolites possess a broad range of activities, which may help in protection against persistent diseases [12] and suggests great potential for the plant as a source of useful phytomedicines. Alkaloids have a wide range of pharmacological activities including antimalarial, antiasthma, anticancer, antiarrhythmic, antibacterial and antihyperglycemic activities [13, 14]. Flavonoids and resins might be responsible for its use as anti-inflammatory recipe in Chinese folkloric medicine as some flavonoids have anti-inflammatory effect on both acute and chronic inflammation [19]. The presence of tannins have astringent properties, which accelerate the healing of wounds and inflamed mucous membrane due to their physiological activities such as anti-oxidant, antimicrobial and anti-inflammatory properties [16]. Steroids have been described to have antibacterial properties [17]. Phenols are largest group of plant metabolites, which have many biological properties such as anti-apoptosis, anti-ageing, anticarcinogen, anti-inflammation and cell proliferating activities [18]. Terpenoids exhibit various important pharmacological activities i.e., antiinflammatory, anticancer, antimalarial, inhibition of cholesterol synthesis, antiviral and antibacterial activities [19]. Plant containing saponins are believed to have antioxidant, anti-cancer, anti-inflammatory, and anti-viral properties. Also have a wide range of medicinal applications [20]. The result from this work has revealed the medicinal potential of these plants in the treatment of bacterial diseases.

Conclusion
The present study revealed that, the extracts of Barleria acuminata leaf was rich in medicinally important class of phytochemical compounds like alkaloids, flavonoids, tannins, steroids, phenols, terpenoids and saponin. Also, the extracts of the plant showed significant antibacterial activities against the bacterial strains.

References