

International Journal of Scientific Research and Reviews

Preliminary Phytochemical Investigation and Antibacterial Activity of Barleria Cristata Linn

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ABSTRACT

The present study was conducted to explore the preliminary phytochemicals and assess the antibacterial activity of *Barleria cristata* leaf extracts. Leaf extracts were prepared from powdered plant materials with various solvents such as acetone, aqueous, dimethyl ether, chloroform and ethanol using Soxhlet apparatus. The qualitative analysis of phytochemicals in the leaf extracts showed positive for alkaloids, vitamin C, flavonoids, tannins, steroids, phenols, terpenoids, glycosides and saponin. Antibacterial activities of the extracts were evaluated by agar well diffusion method. The extracts exhibited significant effects against all bacterial pathogens. The ethanol extracts of leaf showed more inhibitory activity followed by chloroform extract. This present study supports the traditional use of *B. cristata* 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: Phytochemicals, Antibacterial activity, *Barleria cristata*, Traditional plants.

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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 drugs¹. Medicinal plants are widely used for the treatment of human diseases all over the world because they contain components with therapeutic value². 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 infection³. 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 industry⁴. There are many reports on the use of medicinal plants traditionally used by either tribal people or indigenous population⁵. 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 drugs⁶. 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 compounds⁷.

Barleria cristata L. is a shrub found widely in subtropical Himalaya, Sikkim, Central and South India. It has various medicinal and therapeutic uses. Different parts of *Barleria cristata* L. have been used in the treatment of various diseases like anemia, toothache and cough. Root and leaves are used in the treatment of swelling and inflammation⁸. The present study deals with the preliminary phytochemical screening and invitro antibacterial activity of the plant extracts of *Barleria cristata* L.

MATERIALS AND METHODS

Plant sample

Barleria cristata L. was collected from Kanyakumari district, Tamil Nadu, India. The collected leaves were washed in running tap water to remove dust particles, shade dried at room temperature and ground into fine powder using electric chopper.

Preparation of plant extract

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 saponins⁹.

Antibacterial assay

Antibacterial activity of the plant extracts were determined by agar well diffusion method^{10, 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 pneumoniae*, *Proteus mirabilis*, *Salmonella typhi* and *Shigella dysenteriae*. Briefly, fresh bacterial cultures of 0.1 ml having 10⁸ colony forming unit were spread onto 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. Antimicrobial activity was evidenced by the presence of clear inhibition zone around the well and the diameter of this zone was measured with help of Antibiotic Zone Scale-C.

RESULT

Phytochemical screening

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

Table 1: Phytochemical screening of *B. cristata* (L.) leaf extracts

Phytochemical Constituents	Presence (+) or Absence (-) in different extracts				
	Acetone	Chloroform	D. ether	Ethanol	D. water
Alkaloids	-	+	-	+	+
Vitamin C	-	-	-	-	+
Flavonoids	+	+	-	+	-
Tannins	-	-	+	-	+
Steroids	+	-	-	+	+
Phenols	+	-	+	+	-
Phlobatannins	-	-	-	-	-
Terpenoids	+	+	+	+	+
Glycosides	-	-	-	+	-
Saponins	+			+	+

Antimicrobial activity

Antibacterial activities of the extracts were evaluated by a zone of inhibition and the values are measured in mm. The acetone extract of the plant showed considerable inhibition activity on *B. cereus*, *B. subtilis* (14 mm), *E. faecalis* (13 mm), *K. pneumoniae* (17 mm), *S. typhi* (16 mm) & *S. epidermidis* (9 mm); chloroform extract on *K. pneumoniae* (16 mm), *E. coli*, *P. mirabilis* (15 mm), *B. cereus*, *E. faecalis* (14 mm), *B. subtilis* (13 mm), *S. dysenteriae* (11 mm) & *S. epidermidis* (9 mm); ethanol extract on *K. pneumoniae* (14 mm), *E. coli* (13 mm), *S. typhi* (13 mm), *B. cereus*, *B. subtilis* (12 mm), *S. aureus* (11 mm), *E. faecalis* (9 mm) & *P. mirabilis* (8 mm); and water extract on *K. pneumoniae* (14 mm), *S. typhi* (12 mm) & *B. cereus* (8 mm) (Fig. 1).

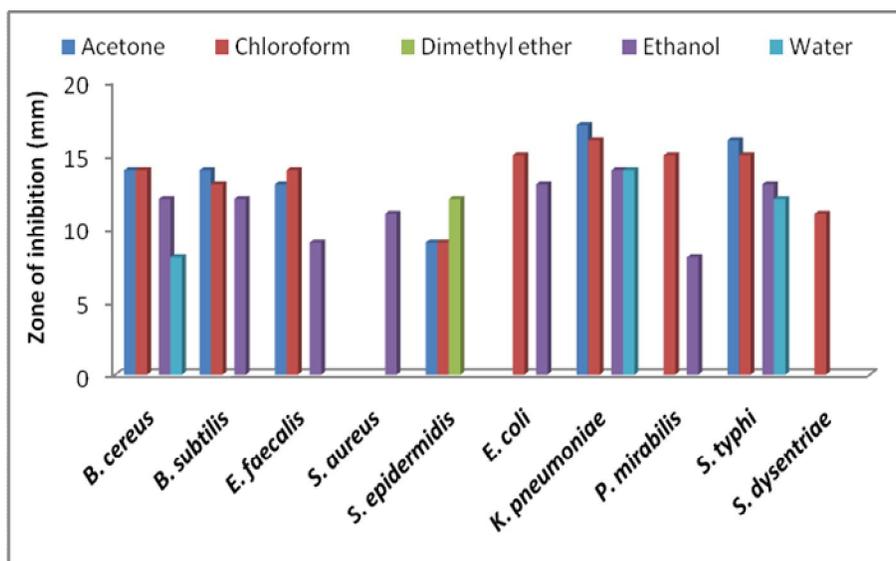


Figure 1: Antimicrobial activity of *B. cristata* (L.) leaf extracts

DISCUSSION

The preliminary phytochemical investigation of the medicinal plant *B. cristata* (L.) leaf extracts revealed the presence of major secondary metabolites such as alkaloids, flavonoids, tannins, steroids, phenols, terpenoids, glycosides and saponin. Among five solvents used ethanol, aqueous and acetone ethanol shows greatest positive results in phytochemical screening. The entire extracts were tested for antibacterial activity against pathogenic bacteria. Among the plant extracts (solvents), ethanol extracts showed maximum antimicrobial activity against both bacterial and fungal pathogens followed by chloroform, acetone and aqueous extract, whereas the dimethyl ether extract showed very less or no growth inhibition against the tested bacterial pathogens. In this assay, both Gram positive and Gram-negative bacterial pathogens were inhibited by the plant extracts. The present investigation was supported by many literatures¹²⁻¹⁴.

The phytochemical constituents of the plant products serve as a defense mechanism¹⁰. These metabolites possess a broad range of activities, which may help in protection against persistent diseases¹⁵ and suggests great potential for the plant as a source of useful phytomedicines. For instance, 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 properties¹⁶. Alkaloids have a wide range of pharmacological activities including antimalarial, antiasthma, anticancer, antiarrhythmic, antibacterial and antihyperglycemic activities^{17, 18}. Flavonoids and resins might be responsible for its use as anti-inflammatory recipe in Chinese folkloric medicine as some flavonoids has anti-inflammatory effect on both acute and chronic inflammation¹⁹. Terpenoids exhibit various important pharmacological activities i.e., anti-inflammatory, anticancer, antimalarial, inhibition of cholesterol synthesis, antiviral and antibacterial activities²⁰. Steroids have been described to have antibacterial properties²¹. Phenols are largest group of plant metabolites, which have many biological properties such as antiapoptosis, antiageing, anticarcinogen, anti-inflammation and cell proliferating activities²². Plant containing saponins are believed to have antioxidant, anti-cancer, anti-inflammatory, and anti-viral properties. Also have a wide range of medicinal applications²³. 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 cristata* leaf was rich in medicinally important class of phytochemical compounds like alkaloids, vitamin C, flavonoids, tannins, steroids, phenols, terpenoids, glycosides and saponin. Also, the extracts of the plant showed significant antibacterial activities against the bacterial strains.

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