

Research Article



An Investigation on Antioxidant and Antimicrobial Activities of Steroidal Alkaloid Isolated from *Buxus sempervirens* Leaf

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ABSTRACT

The study was conducted to examine the *in vitro* antimicrobial activity and antioxidant activity of steroidal alkaloid from *Buxus sempervirens*. Well diffusion technique was used for *in vitro* antimicrobial screening against bacteria and fungi. The extract showed maximum zone of inhibition against *Bacillus cereus* and *Alternaria alternate* and the isolated bioactive compound possessed more antioxidant activity at a minimum concentration.

Keywords: Steroidal Alkaloid, *Buxus sempervirens*, Antimicrobial and Anti oxidant activity.

INTRODUCTION

In recent years, the growing demand for herbal product has led to a quantum jump in volume of plant materials traded within and across the countries. The secondary metabolites, previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents¹. Thus it is anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of the bacterial infections².

Approximately 20% of the plants found in the world have been submitted to pharmacological or biological tests³. The systemic screening of antimicrobial plant extracts represents a continuous effort to find new compounds with the potential to act against multi-resistant pathogenic bacteria and fungi. A special feature of plants is their capacity to produce a large number of organic chemicals of high structural diversity called secondary metabolites⁴ (Evans *et al.*, 1986), which are divided into different categories based on their mechanism of function like chemotherapeutic, bacteriostatic, bactericidal and antimicrobial⁵.

Nowadays multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease. In addition to this problem are sometimes associated with adverse effects on the host including hypersensitivity, immune suppression and allergic reaction this situation forced scientists to search for new antimicrobial substances⁶. The safety of synthetic compounds, however, has been cause of concern and has stimulated the evaluation of the effectiveness of natural compounds, or extracts with potent antioxidative and antibacterial activities⁷. Hence, the study was intended to examine the potential of bioactive compounds for its antimicrobial and *in vitro* antioxidant activity.

MATERIALS AND METHODS

Collection of Sample

The Fresh elder leaves of the plant *Buxus sempervirens* was collected from Thanjavur. The collected plant materials were washed thrice in tap water to remove the adhering the soil Particles and one time washed in the sterile distilled water and allowed to shade dried for 15 days.

Preparation of Sample

The extraction of crude extract, 100g of leaf powder was taken in 1 liter capacity round bottom flask and mixed with butanol at 48hrs sequentially. The extracts, were filtered using Whatmann 40 filter paper and concentrated to prepare the crude extract.

Isolation of Steroidal Alkaloid by Thin Layer Chromatography⁸

A part of sample extract was dissolved in Pet. Ether solvent and subjected to TLC studies by using silica gel G as stationary phase. The solvent system was chloroform: methanol: water (14:6:1), the chromatogram is sprayed in Dragendorff's reagent. TLC showed well-separated spots confirmed that extract contains Steroidal alkaloid.

Pharmacological Studies by *In Vitro* Methods

*Antioxidant Activity*⁹

1 ml of plant extract and isolated compound were mixed with phosphate buffer (2.5 ml 0.2 M, pH 6.6) and potassium ferric cyanide (2.5 ml). The mixture was incubated at 50C for 20 minutes. A portion (2.5 ml) of Trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5ml) was mixed with distilled water (2.5ml) and Ferric chloride (0.5ml, 0.1%) and absorbance measured at 700nm. Increased absorbance of



the reaction mixture indicates stronger reducing power.

The activity was compared with ascorbic acid standard.

Calculation

$$\text{Percentage scavenging activity} = \frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}} \times 100$$

Where A_{control} is the absorbance of the control. A_{test} is the absorbance in the presence of the sample.

Antimicrobial Activity by Well Diffusion Method¹⁰

The microbial cultures such as *Pseudomonas aeruginosa*, *Bacillus cereus*, *Vibrio cholera*, *Salmonella typhii*, *E. coli*, *Rhizactonia solani*, *Fusarium udum*, *Macrophomina phaseolina*, *Alternaria alternata* and *Sclerotium roysii* were collected from Amphigene research lab, Thanjavur and sub cultured using Nutrient agar medium. The antimicrobial potential of both the isolated compound and leaf extract were evaluated according to their zone of inhibition against various pathogens and the results (zone of inhibition) were observed.

RESULT AND DISCUSSION

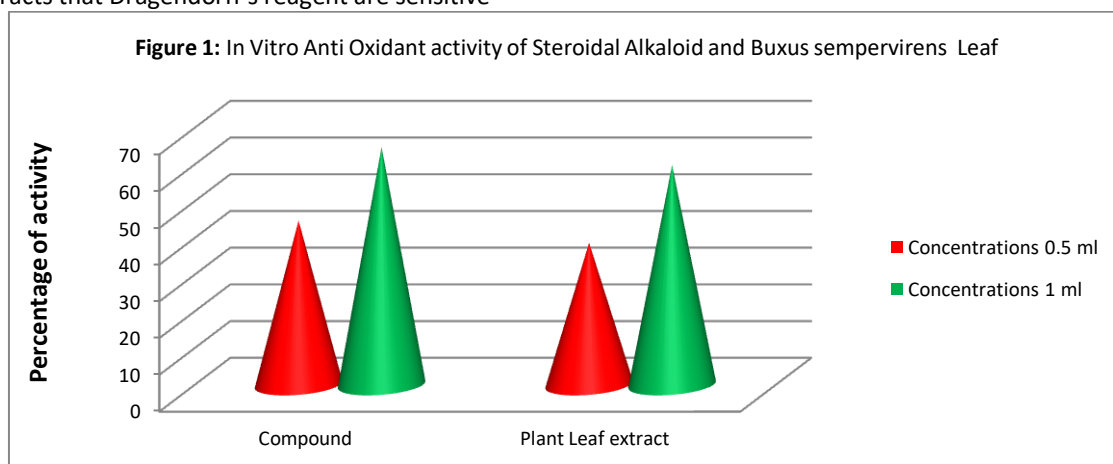
Isolation of Steroidal alkaloid by TLC

The bioactive compound steroidal alkaloid **was** isolated from the *B. sempervirens* with the rf value was 0.88 respectively. The separation of alkaloids showed in butanol's extracts that Dragendorff's reagent are sensitive

for concentrations higher only, where gave good results with the compounds in the *Solanum elaeagnifolium*¹¹. In this study also butanol extract gave the good result for the isolation of steroidal alkaloid using Dragendorff's reagent.

In Vitro Antioxidant activity

The antioxidant property of the both compound and plant leaf extract was analyzed by Power reducing assay. The compound showed potent free radical scavenging activity due to the presence of alkaloid. The compound contains 44.71 and 64.71% of antioxidant activity at 0.5 and 1 ml concentration respectively. Whereas the plant leaf extract possessed 38.68 and 59.81% of activity at the same concentration (fig 1). High ROS can cause oxidative stress in cells and antioxidants are important in controlling damage caused by ROS production. Soon *et al.*, 2013 evaluated the efficacy of these alkaloids in reducing H₂O₂-induced oxidative stress by monitoring 5-(and-6)-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate (CM-DCFDA) fluorescence¹².

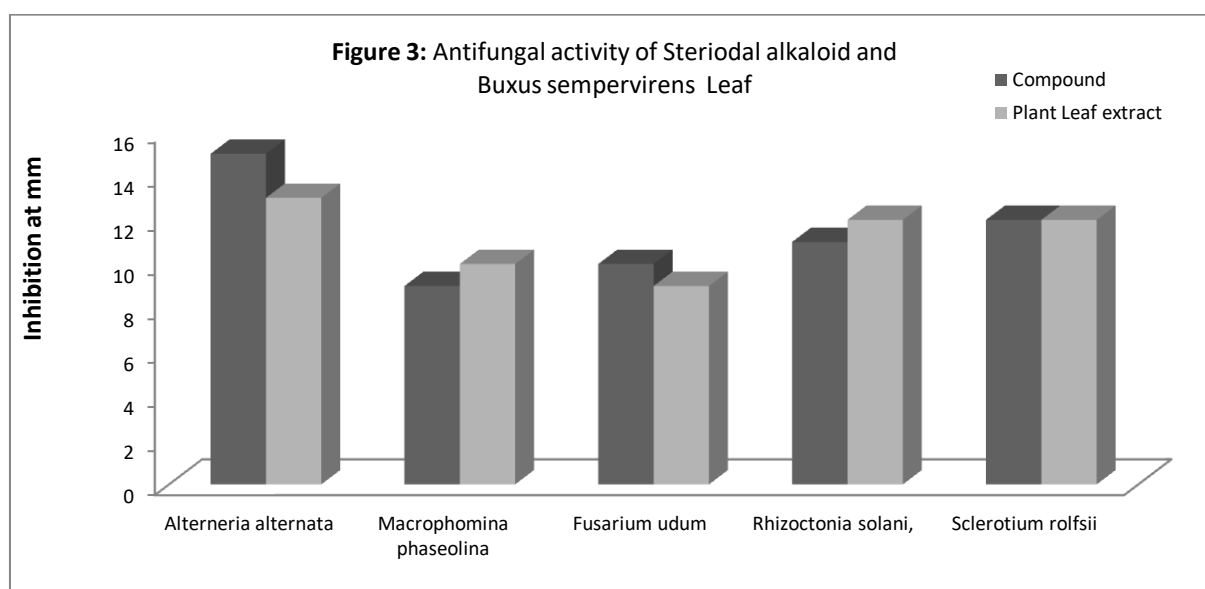
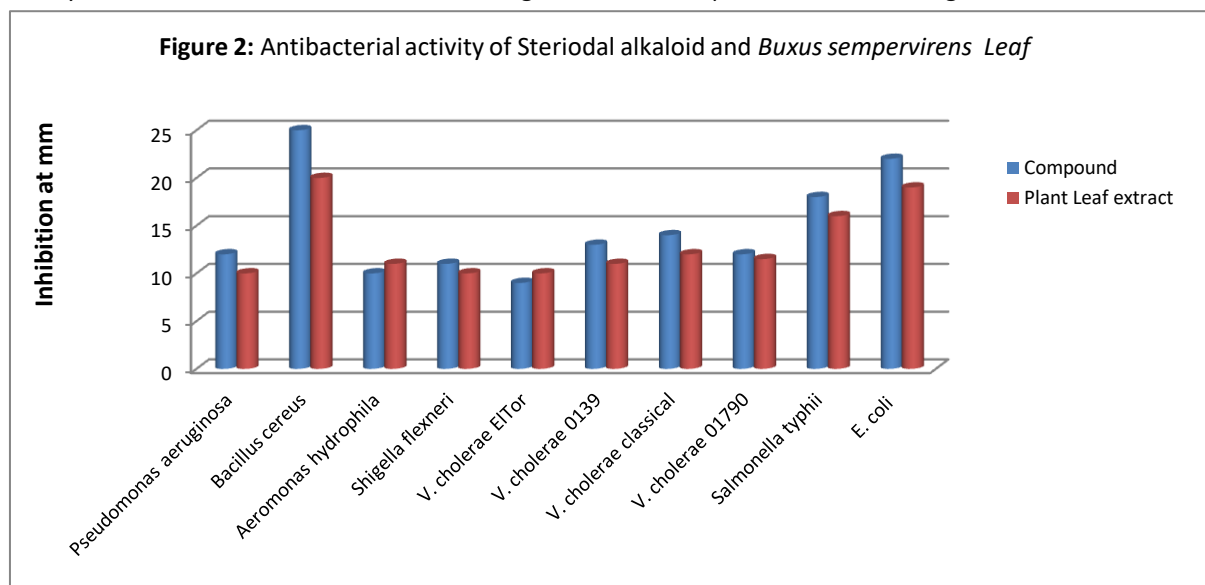


In-Vitro Anti-microbial activity

The antimicrobial property of steroidal alkaloid and the leaf extract were tested against ten bacterial species and five fungal species by well diffusion method. The zone of inhibition was assessed and the plates were kept at room temperature for 24–48 hours. The microbial species namely, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Aeromonas hydrophila*, *Shigella flexneri*, *V. cholerae* EITor, *V. cholerae* O139, *Salmonella typhii*, *V. cholerae* classical, *V. cholerae* O1790 and *E. coli* (ETEC). And also some selected fungus species such as *Rhizoctonia solani*, *Fusarium udum*, *Macrophomina phaseolina*, *Alternaria alternata* and *Sclerotium rolfsii* were used to evaluate the inhibitory activity.

The study showed the maximum antibacterial activity was recorded for steroidal alkaloid against *P2-Bacillus cereus* (25mm) whereas minimum inhibitory activity was recorded *V. cholerae* EITor (9mm) respectively (fig 2), Likewise, the maximum inhibitory activity was observed against *Alternaria alternata* (15mm) and minimum activity was noted against *Macrophomina phaseolina* (9mm), whereas low inhibition zone present in against *Fusarium udum*, and *Sclerotium rolfsii* respectively. The antibacterial and antifungal activity in vitro of crude alkaloids from *Anabasis articulata* using the disc diffusion method and concluded that alkaloids might be responsible for its anticandidal activity¹³. The antibacterial and synergistic activity of alkaloid extracts of *B. microphylla* leaves, stems and roots used either

individually or in combination with antibiotics against Gram-positive and Gram-negative bacteria¹⁴.



CONCLUSION

The study explored that the lead bioactive compound responsible for antibacterial and antioxidant activity from this plant. Further study will be conducted for the anticancer property of the isolated compound from the plant.

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