Research Article

# Preliminary Phytochemical Analysis and Antifungal Activity of Methanolic Extract of Leaves of *Actinodaphne bourdillonii* Gamble

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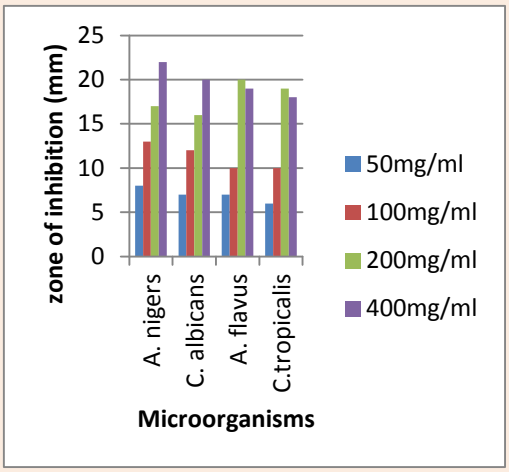
## Abstract

The aim of this study is to screen the phytochemicals present in the methanolic extract of leaves of *Actinodaphne bourdillonii* Gamble and its antifungal activity. The in vitro antifungal evaluation was carried out by agar well diffusion method against four fungal strains *Aspergillus niger, Aspergillus flavus, Candida albicans and Candida tropicalis*. Preliminary phytochemical studies revealed the presence of alkaloids, cardiac glycosides, saponin, tannin, terpenoids, and phlobatannins in the methanolic extract of leaves of A. bourdillonii Gamble. Antifungal activity of the extract was tested with four different concentrations (50, 100,200 400 mg/ml). Highest antifungal activity was found in *Aspergillus niger* while least antifungal activity was in Candida tropicalis.




**Keywords:** Phytochemicals, *Actinodaphne bourdillonii,* Antifungal activity, methanolic extract, fungal strain.

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## Introduction

Herbal medicine is the oldest form of healthcare known to mankind. Herbal had been used by all cultures throughout history. Plants are a major source of therapeutic remedies for infectious diseases in all over the world [8]. Plants synthesize a dazzling array of organic chemical components called secondary metabolites, whose function has been debated. Many secondary metabolites are "Antibiotic" protecting the plants against fungi, bacteria, animals, and even other plants. Of 2, 50,000 known species of higher plants, nearly 35,000 species has been used by the people for various medicinal purposes. Phytochemicals tannins, terpenoids, alkaloids, and flavonoids are known to possess antimicrobial properties [1].

Microorganisms have become resistant and also occasionally associated with hypersensitivity, immunosuppressant and allergic reactions if the antibiotics are used continuously [6]. Therefore, it is essential to develop alternative antimicrobial drugs for the treatment of various infectious diseases. The present study evaluates the in vitro antifungal activity of methanolic extracts of leaves of *A. bourdillonii* against four fungal strains namely *Aspergillus niger, Aspergillus flavus, Candida albicans* and *Candida tropicalis* and qualitative analysis of phytochemicals.

## Materials and Methods

### Collection of Samples

Fresh *A. bourdillonii* leaves were collected from Munnar, (Lat 10°1'22"N; Long 77°2'22"E) Idukki district Kerala, India. The plant was authenticated by the Rapinat Herbarium and Centre for Molecular Systematic, St.Joseph's College (Campus) Tiruchirappalli 620020, Tamil Nadu, India.

**Preparation of plant extracts [3]**

The collected leaves of the plant were shade dried and powdered. 10g of the powder was macerated in 100ml of methanol and placed in a mechanical shaker for 48 hours. The extract was filtered using No.1 Wattman filter paper. The filtrate was concentrated using rotary evaporator. The extract was stored in the refrigerator for further analysis.

**Preliminary phytochemical screening**

Qualitative phytochemical analysis of *A. bourdillonii* was carried out by using standard procedures to identify the constituents as described [7,2,5]

**Test for tannins:**

One ml of the leaf extract was treated with few drops of 1% ferric chloride solution and observed for brownish green or a blue-black coloration which indicates the presence of tannins.

**Test for saponins**:

Two ml of distilled water was added to 2ml of the leaf extract and shacked well and observed for frothing which indicates the presence of saponins.

**Test for cardiac glycoside**

Five ml of leaf extract was treated with 2ml of glacial acetic acid containing 1 drop of ferric chloride. This was under layered with 1ml of concentrated sulphuric acid. Formation of brown ring at interphase indicated the presence of deoxy sugar which is the characteristic of cardiac glycoside.

**Test for phlobatannins**

One ml of leaf extract was treated with few drops of 1% hydrochloric acid and boiled. The formation of red precipitate indicates the presence of phlobatannins.

**Test for terpenoids**

Five ml of leaf extract was mixed with 2ml of chloroform and then 3ml of concentrated sulfuric acid was added carefully. A reddish brown coloration of the interface shows the presence of terpenoids

**Test for flavonoids**

Two ml of leaf extract was mixed with 2ml of sodium hydroxide. Appearance of yellow coloration indicates the presence of flavonoids.

**Alkaloids (**Mayer's Test)

A small quantity of the extract was treated with few drops of dilute hydrochloric acid and filtered. The filtrate was tested with alkaloid Mayer's reagent. Formation of cream precipitate indicates the presence of alkaloids.

**Test for phytosterols** *(Libermann-Buchard's test)*

5 ml of extract was mixed with 2 ml of acetic anhydride and 1 or 2 drop of concentrated sulphuric acid was added slowly along the sides of the test tubes. An array of color change shows the presence of phytosterols.

**Microorganism used**

The fungal microorganisms used in the present study include *Aspergillus niger, Aspergillus flavus, Candida albicans and Candida tropicalis*

**Antifungal activity**

Antifungal activity was screened by agar well diffusion method adapted from [4] 3.95g of Potato Dextrose agar was dissolved in 100 ml of distilled water and sterilized in an autoclave at 15lbs for 15mins. The sterilized media were poured into sterile petri dishes. This was allowed to solidify and spread the fungal spores on petri plates and holes per plate were made in the medium by using a sterile cork-borer of 5 mm diameter. Thereafter, the wells were filled with the extract solution at varying concentrations (50, 100,200 400 mg/ml). This was done in duplicate and plates were incubated at suitable temperature of 27ºC for 1-3 days. The plates were observed for zone of inhibition (mm).

## Results and Discussion

The results of the preliminary phytochemical test of methanolic extract of the leaves shows presence of Alkaloids, Cardiac glycosides, Saponin, Tannin, Terpenoids, Phlobatannins and reported in the Table 1. The physicochemical parameters of leaves of *A. bourdillonii,* pH – 6.1, total ash content 6.5%, water soluble ash 2.91%, acid insoluble ash 1.2% have already been studied by the authors. The antifungal activity Table 2, Figure 1 results reveal that the extracts exhibit a significant antifungal activity when compared with standard drug Ketoconazole. The highest antifungal activity was found in *Aspergillus nigers* (22 mm) followed by *Candida albicans* (20mm), *Aspergillus flavus* (19mm) and *Candida tropicalis* (18mm).

**Table 1** Phytochemical constituents present methanolic extract of leaves of *Actinodaphne bourdillonii*

| S.NO | Parameters | Result |
|---|---|---|
| **1** | Phlobatannins | Present |
| **2** | Terpenoids | Present |
| **3** | Phytosterols | Absent |
| **4** | Flavonoids | Absent |
| **5** | Tannin | Present |
| **6** | Saponin | Present |
| **7** | Cardiac glycosides | Present |
| **8** | Alkaloids | Present |

**Table 2** Antifungal activity of leaves of *Actinodaphne bourdillonii* using Disc diffusion method

| Concentration (mg/ml) | *Aspergillus nigers* | *Candida albicans* | *Aspergillus flavus* | *Candida tropicalis* |
|---|---|---|---|---|
| **50** | 8 | 7 | 7 | 6 |
| **100** | 13 | 12 | 10 | 10 |

| | | | | |
|---|---|---|---|---|
| **200** | 17 | 16 | 15 | 13 |
| **400** | 22 | 20 | 19 | 18 |
| **Standard Ketoconazole** | 21 | 18 | 20 | 20 |

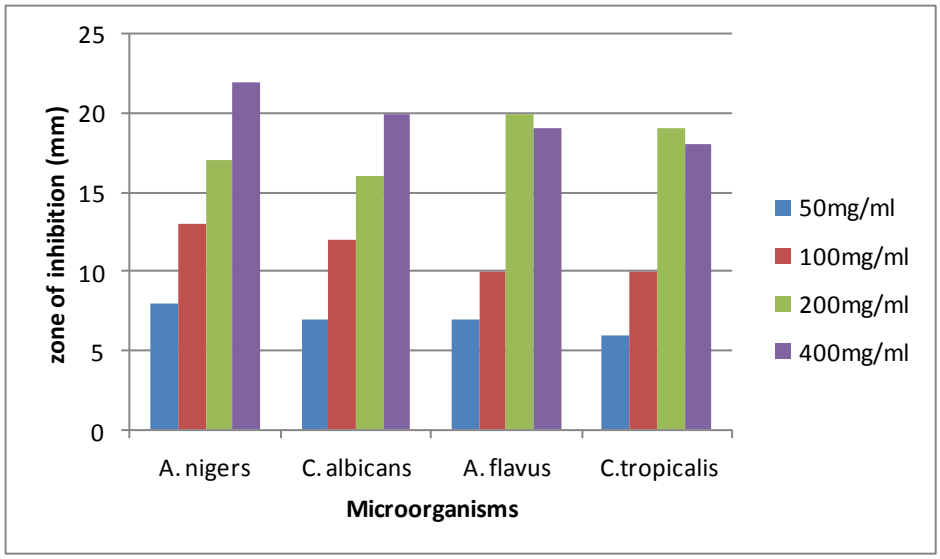


**Figure 2** Antifungal activities of leaves of Actinodaphne *bourdillonii* using Disc diffusion method

## Conclusion

From the findings of this study, phytochemicals are present in the methanolic extracts of leaves of *A. bourdillonii* and it possesses strong antifungal activity. Further purification of active principles in the extract will lead to the formation of new drugs which can be used for the treatment of various infectious diseases.

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