IJRAR.ORG E-ISSN: 2348-1269, P-ISSN: 2349-5138

INTERNATIONAL JOURNAL OF RESEARCH AND ANALYTICAL REVIEWS (IJRAR) | IJRAR.ORG

An International Open Access, Peer-reviewed, Refereed Journal

PHYTOCHEMICAL PROFILING, ANTIMICROBIAL AND ANTIOXIDANT POTENTIAL OF *ACTINODAPHNE BOURDILLONII* GAMBLE LEAF EXTRACTS.

[1*] **SR. SINDHU K. K,** [2] **DIYA JOSEPH,** [2] **ASWATHI.B**

1.ASSISTANT PROFESSOR 2. RESEARCH SCHOLAR 2. POST GRADUATE

POST GRADUATE AND RESEARCH DEPARTMENT OF BOTANY

MERCY COLLEGE PALAKKAD

ABSTRACT

The plant kingdom is an abundant source of extraordinary compounds known as primary and secondary metabolites that are physiologically active and function as antimicrobials. The present study evaluates the phytochemical components, antimicrobial activities, and antioxidant properties of the leaf extract of *Actinodaphne bourdillonii*. Various solvents were used to extract phytochemicals from leaves including petroleum ether, acetone, methanol, and water. The methanolic extract showed the highest antioxidant activity, assessed by the DPPH method. Antibacterial activity was tested in vitro by the disc diffusion method against five bacterial strains, including Bacillus cereus, Salmonella typhi, Escherichia coli, Staphylococcus aureus, and Enterobacter aerogenes, with the acetone leaf extract displaying the highest inhibition zone (16 ± 0.021 mm) against E. coli. The antifungal activity was evaluated using the fungal strain Candida albicans. This study indicates that the leaf extract of *A. bourdillonii* possesses antibacterial and antifungal properties, highlighting its potential as an effective medicinal agent.

Keywords: *Actinodaphne bourdillonii*, phytochemistry, DPPH, antimicrobial, antioxidant

INTROCTION

Plants are valuable assets in the global fight against serious diseases. In developing nations, traditional medical practices, especially medicinal plants, are essential for addressing basic healthcare needs (Thirumurugan et al., 2010). Plants naturally contain chemical substances called phytochemicals, which are biologically active (Hasler & Blumberg, 1999). Phytochemicals can be stored in various plant parts, including the roots, stems, leaves, flowers, fruits, and seeds (Costa et al., 1999). These compounds enhance the color, flavor, and fragrance of the plants while protecting against several diseases (Gibson et al., 1998).

Actinodaphne is a genus of about 100 species of evergreen trees and shrubs that occur mainly in tropical-subtropical Asia and are an important component of tropical forests. Many of the species of *Actinodaphne* are used as a traditional medicine against various diseases. *A. hookeri* Meissn is traditionally used to cure diabetes and urinary tract diseases, which are more widespread in eastern India and Chhattisgarh. Its main constituents are flavonoids and quercetin-3-rhamnoside (Prajapati et al., 2009). The methanolic bark extract of *A. pruinosa* demonstrated the highest activity on DPPH, ABTS, and phenolic content. In contrast, the bark extracts of *A. macrophylla* demonstrated the best inhibitory activity against mushroom tyrosinase and lipoxygenase assays (Salleh & Ahmad, 2016). *A. angustifolia* is commonly used in folk medicine against urinary disorders and diabetes (Uddin et al., 2020). *A. bourdilloni* is a plant with high medicinal value. The preliminary phytochemical analysis in the methanol extract of *A.bourdilloni* revealed the presence of various phytochemicals such as Terpenoids, phytosterols, flavonoids, Tannins, saponins, and alkaloids (Deepa & Selvakumr, 2015). The present study aims to evaluate the phytochemical components, antimicrobial activities, and antioxidant properties of the leaf extract of *A. bourdillonii* using various solvents.

MATERIALS AND METHODS

Collection of Plant Material

Fresh leaves of *Actinodaphne bourdilloni* were collected from Nelliyampathy, Palakkad, Kerala, and stored in zip-lock bags with appropriate labeling. They were kept in an ice cooler until transported to the laboratory for extraction.

Preparation of Plant Extract

The collected plant material is properly washed with distilled water to remove undesirable materials, dried under shade at room temperature, and then powered using a mixer and grinder. The fine powder of plant material was then extracted with the help of the soxhlet apparatus by using solvents to increase polarity, namely petroleum ether, acetone, methanol, and distilled water, respectively. 30g powdered leaves were added in 100ml of the respective solvents. The time taken by each extraction is 24-48 hours. Then, the extracts were collected, filtered, and kept in the glass containers in the refrigerator for further analysis.

Preliminary Qualitative Phytochemical Screening

The presence or absence of the bioactive phytoconstituents such as alkaloids, flavonoids, glycosides, saponins, starch, Proteins, anthocyanin, amino acids, tannins, phenols, phytosterols, carbohydrates, and terpenoids of the leaf extracts was accessed by standard phytochemical assay methods.

Determination of Antibacterial Activity

Microorganisms used

Bacillus cereus (MTCC 430), *Salmonella typhi* (MTCC 98), *Escherichia coli* (MTCC 452), *Staphylococcus aureus* (MTCC 96), and *Enterobacter aerogenes (*MTCC 2828) were collected from the culture collection Institute of Microbial Technology (IMTECH). The bacteria were sub-cultured on nutrient slants and stored in the refrigerator for further experiments.

Preparation of nutrient agar medium

28g of nutrient agar was weighed and dissolved in 1000 ml of distilled water. Then, the medium was heated until the agar completely dissolved and autoclaved at 121°c at 15 psi for 20 minutes. The medium, Petri dishes, and cotton swabs were kept in the laminar airflow hood and sterilized by UV. After sterilization, the nutrient agar medium was poured into Petri dishes under aseptic conditions. This medium was allowed to solidify for 30 minutes.

Disc diffusion method

Antimicrobial assay was performed by the disc diffusion method. 20 ml of sterile nutrient agar media was poured into sterile Petri dishes under aseptic conditions. Before streaking, the bacterial strains were diluted in sterile distilled water using a sterile inoculation loop. Then, 100 ml of microbial suspension was inoculated into the solidified media and spread over the medium using a sterile cotton swab. The discs, which had been impregnated with different solvents of leaf extract, are placed on the surface of the media. Each petri plate contains six discs. Streptomycin was used as the positive control, and pure solvent was used as the negative control. The plates were incubated for 24 hrs. The clear formation that appeared around the discs indicated antibacterial activity. They were measured after 24 hrs and presented in mm.

Determination of Antifungal Activity

Microorganism used

Candida albicans (MTCC 183) were collected from the culture collection Institute of Microbial Technology (IMTECH). The fungi were sub-cultured on nutrient slants and stored in the refrigerator for further experiments.

Preparation of SDA medium

SDA medium was prepared by adding 4g of dextrose, 1g of peptone, and 1.5g of agar in 100 ml of distilled water in a conical flask and autoclaved at 15 lbs. for 15 mins. The medium is placed in a laminar airflow hood for 20 minutes and exposed to UV light. Under aseptic conditions, the medium is poured into sterile Petri dishes. After 30 minutes, the solidified medium was appropriately kept in the refrigerator to avoid contamination.

Preparation of broth culture

SDB (Sabouraud dextrose broth) was used to culture the fungal strain. 4g of dextrose and 1g of peptone dissolved in 100 ml of distilled water. In a microwave oven, the broth is heated until it turns a translucent golden-yellow color. Then, the broth is sterilized using a pressure cooker for 15 minutes. After that, the broth was again sterilized by UV. Under aseptic conditions, the broth was poured into sterile conical flasks. The fungus, *Candida albicans,* was transferred from the slant into a conical flask containing broth using a sterile inoculation loop. To obtain optimal growth, fungal cultures were incubated for 48 hours in an incubation shaker.

Disc diffusion method.

Using a micropipette,100 µl of broth culture was transferred to the SDA medium containing Petri dishes and streaked under aseptic conditions. 6 mm blank sterile discs were used. Sterile discs were impregnated with extracts from different solvents (petroleum ether, acetone, methanol, and water). Pure solvents were used as the negative control, and streptomycin was used as the positive control. The discs were placed carefully, and the zone of inhibition was calculated by measuring the diameter of the zone after 48 hours.

Determination of Antioxidant Activity

The free radical scavenging property of the various extracts of *A. bourdillonii* [acetone, methanol, petroleum ether, and aqueous extracts] was measured in vitro by the DPPH assay. The stock solutions were prepared freshly (1 mg in 0.1 mL). DPPH solution was then diluted with methanol to achieve an absorbance of approximately 0.98 ± 0.02 at 517 nm, as measured using a spectrophotometer. A 3 mL aliquot of this DPPH solution was then added with 1 mL of the sample at various concentrations (100-500 µg/mL) of extracts, along with a standard solution of ascorbic acid. The reaction mixture was shaken thoroughly and incubated in the dark for 15 minutes at room temperature. Then, the absorbance (Abs) was taken at 517 nm; a 0.1mM methanol solution of DPPH alone served as the control. Methanol served as a blank to set zero. The DPPH assay results were calculated using the following formula:

$$DPPH\ radical\ scavenging\ activity\ \% = \frac{Control\ OD - Sample\ OD}{Control\ OD\ X\ 100}$$

RESULT AND DISCUSSION

Preliminary Qualitative Phytochemical Screening results revealed the presence of a variety of phytoconstituents including alkaloids, proteins, amino acids, phenols, tannins, terpenoids, glycosides, starch, phytosterols and saponins. (Table .1)

Table. 1: Phytochemicals present in different leaf extracts of *A. bourdilloni*

QUALITATIVE PHYTOCHEMICAL ANALYSIS OF THE PLANT EXTRACT						
Sl No.	Phytochemical test	Phytochemicals	Solvent			
			Petroleum ether	Acetone	Methanol	Distilled water
1	Hager's test	Alkaloids	-	-	+	-
2	Biuret test	Protein	-	+	-	+
3	Ninhydrin test	Amino acids	-	+	-	-
4	Ferric chloride test	Phenol	+	+	+	+
5	Lead acetate test	Tannin	-	+	+	+
6	Lead acetate test	Flavonoid	-	-	-	-
8	Salkowski's test	Terpenoids	-	-	+	+
9	Keller Killani test	Glycoside	-	-	+	-
10	Fehling's test	Carbohydrates	-	-	-	-
11	Iodine test	Starch	-	-	+	-
12	Liebermann Burchard's test	Phytosterol	-	+	-	-
13	Foam test	Saponins	-	+	+	+
14	HCl test	Anthocyanins	-	-	-	-

**(+) sign indicates the presence of phytochemical compound and (-) indicates the absence of phytochemical compound.

IN VITRO ANTIBACTERIAL ACTIVITY

Using the agar disc diffusion assay, the antibacterial properties of petroleum ether, acetone, methanol, and distilled water were evaluated against five distinct bacterial strains. An extract containing 100 µl was injected into each disc. Significant antibacterial activity was demonstrated by all solvent plant extracts against different test organism strains (Table. 2, Figure. 1) .

Methanol extract has the highest antibacterial activity against *Salmonella typhi* with a zone of inhibition measuring 15±0.00 mm in diameter. Petroleum ether has no activity against these five bacterial strains. Zone of inhibition against *S. aureus* was seen to have 13±0.001 mm diameter in acetone leaf extract, 16±0.021 mm against *E. coli*, whereas *B. cereus* and *E. aerogenes* showed a maximum inhibition zone of 11±0.010mm diameter. Methanol leaf extract showed a 15±0.00 mm zone of inhibition against *S. typhi*, 12±0.021mm diameter against B. *cereus* and *E. coli*. *S. aureus* and *E. aerogenes* with a zone of inhibition of 13±0.001mm, respectively. With a diameter of 7±0.01 mm, the distilled water leaf extract zone of inhibition against *E. aerogenes and E. coli*, whereas no activity against *S. aureus, S. typhi*, and *B. cereus*. The disk that had 100 µl of streptomycin was used as the positive control, whereas the disc that contained 100 µl of solvent alone was used as the negative control. Inhibition zone against streptomycin, the positive control, ranged from 17 to 22 mm in diameter, with the lowest inhibition zone against S. aureus and the highest against *E. coli*. When tested against test organisms, the negative control has no antibacterial action.

Table.2: An overview of inhibitory efficacy of leaf extract of *A. bourdillonii* against test organisms.

Sl No	Bacterial strain	Zone of inhibition (mm ±SD) in different plant extracts				+ve control	-ve control
		Petroleum ether	Acetone	Methanol	Water	Streptomycin	Solvent alone
1	*Staphylococcus aureus*	-	13±0.001	13±0.001	-	20±0.020	-
2	*Salmonella typhi*	-	13±0.002	15±0.00	-	20±0.002	-
3	*Bacillus cereus*	-	11±0.010	12±0.021	-	20±0.012	-
4	*Enterobacter aerogenes*	-	11±0.011	13±0.001	7±0.01	20±0.011	-
5	*Escherichia coli*	-	16±0.021	12±0.012	7±0.001	20±0.021	-

***(-)** sign indicates the absence of inhibitory activity against the test organism.

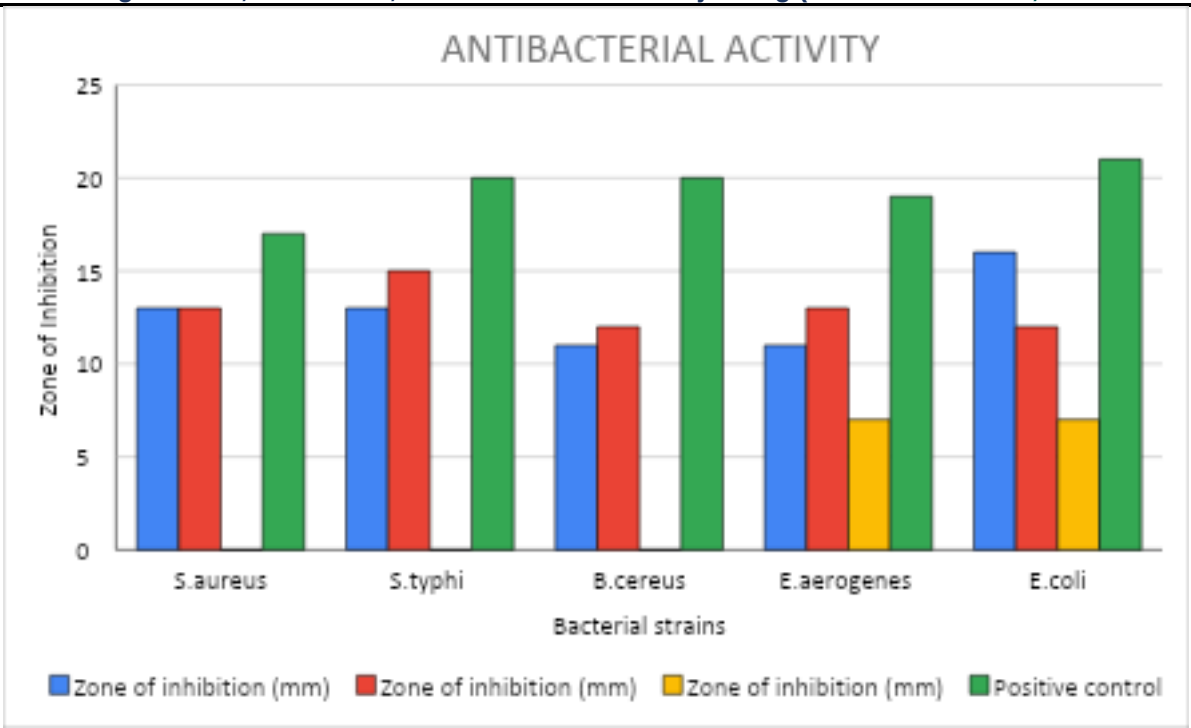

Fig.1 Antibacterial activity of four different solvent extracts of *A. bourdillonii*

IN VITRO ANTIFUNGAL ACTIVITY

Using the agar disc diffusion method, the four distinct plant extracts- petroleum ether, acetone, methanol, and distilled water—showed strong antifungal activity against the test organism, Candida albicans (Table. 3, figure. 2). With a zone of inhibition against the test organism of 13± 0.02 mm in diameter, the methanol leaf extract of *A.bourdilloni* exhibited the highest antifungal activity, followed by the acetone leaf extract with an inhibition zone of 9 ± 0.04 mm diameter. Zone of inhibition with a diameter of 1± 0.03 mm was expressed by petroleum ether and distilled water leaf extract. The positive control, itraconazole (100 µl), exhibited a 2±0.003 mm zone of inhibition. The solvent by itself was used as a negative control to demonstrate the lack of antifungal action.

Table. 3 An overview of inhibitory efficacy of leaf extract of *A. bourdillonii* against test organism.

Fungus	Zone of inhibition (mm) in different plant extracts				+ve control	-ve control
Candida albicans	Petroleum ether	Acetone	Methanol	Water	Itraconazole	Solvent alone
	1± 0.03	9 ± 0.04	13± 0.02	1± 0.03	2±0.003	-

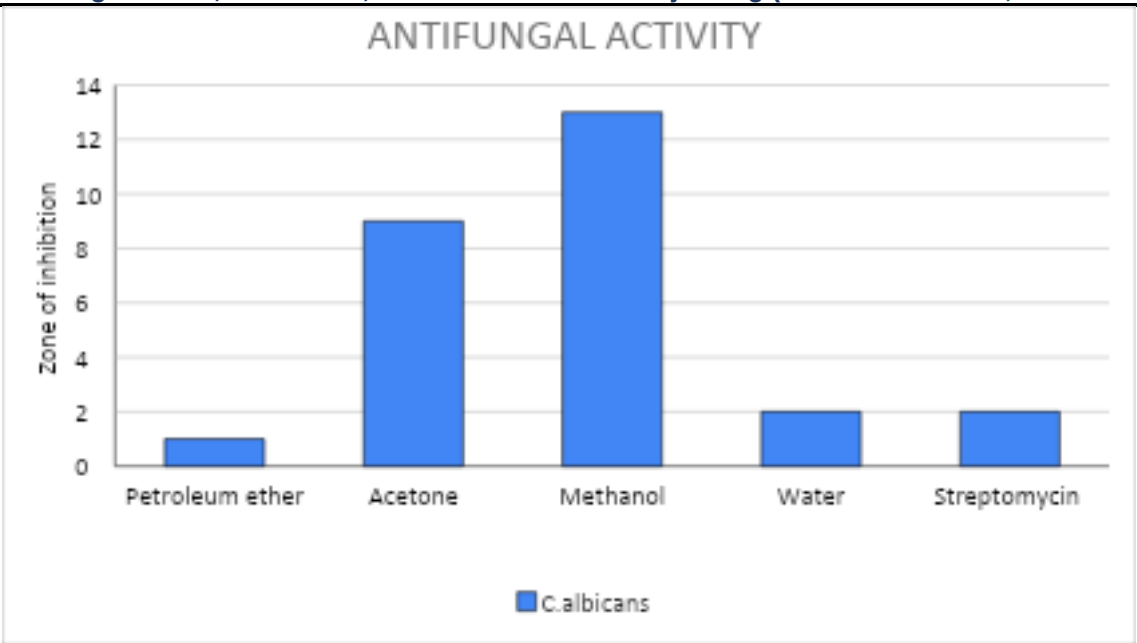

Fig.2 Antifungal activity of four different solvent extracts of *A. bourdillonii*

IN VITRO ANTIOXIDANT ACTIVITY

Improving trend with DPPH radical scavenging activity of the sample was observed, which was truly convincing comparable to that of ascorbic acid (Table. 4) In vitro antioxidant activity of the extracts displayed dose dependency – increase in scavenging activity with increase in concentrations of the sample. Experiments were done in triplicates and the mean values were tabulated. Methanolic extract of *A. bourdillonii* proved outstanding due to its lower IC50 value – 70.6µg/mL, relatively equivalent to that of ascorbic acid standard. Acetone extract (IC_{50} value – 254µg/mL) of *A. bourdillonii* exhibited vibrant antioxidative property, followed by petroleum ether extract (IC_{50} value – 306µg/mL**)** and aqueous extract (IC_{50} value – 342µg/mL) of the same when compared to ascorbic acid with IC50 value of 69µg/Ml.

Table. 4 An overview DPPH radical scavenging activity of Samples

Sample	**IC_{50} ($\frac{\mu g}{mL}$)**
Ascorbic acid	69
Methanol Extract	70.6
Acetone Extract	254
Petroleum ether Extract	314
Water Extract	342

CONCLUSION

Phytochemicals are the bioactive compounds synthesized by plants for their protection. They have the potential to act as potent antioxidants and antimicrobial agents. Based on the study's findings, leaf extracts of *Actinodaphne s*howed the production of phytochemicals that can be used in the pharmaceutical industry. Antimicrobial resistance represents a significant global public health challenge. Exploiting plant sources to identify new and effective antibacterial phytocomponents will be motivated by the need to discover novel antibacterial agents with unique modes of action. Plant-based antimicrobial compounds have enormous

therapeutic potential as they can serve the purpose without any side effects that are often associated with synthetic antimicrobials. Based on the study's findings, it is possible to conclude that *Actinodaphne* leaf extract can prevent the growth of bacteria. The extract's active components will need to be further purified to create new medications that can be utilized to treat a variety of infectious disorders. According to the study's findings, the leaves of this plant may be a good source of naturally occurring antioxidants, which may be very useful as therapeutic agents to stop or slow the onset of age-related degenerative diseases and the aging process. However, more research is needed to isolate and characterize the antioxidant components fully.

REFERENCES

1. Costa, M. A., Xia, Z. Q., Davin, L. B., & Lewis, N. G. (1999). Toward engineering the metabolic pathways of cancer-preventing lignans in cereal grains and other crops. Phytochemicals in human health protection, nutrition, and plant defense, 67-87.
2. Deepa, R., & Selvakumar, S. (2015). Preliminary Phytochemical Analysis and Antifungal Activity of Methanolic Extract of Leaves of *Actinodaphne bourdillonii* Gamble. *Chemical Science review and Letters*, 4(13),148-152.
3. Gibson, E. L., Wardle, J., & Watts, C. J. (1998). Fruit and vegetable consumption, nutritional knowledge and beliefs in mothers and children. Appetite, 31(2), 205-228.
4. Hasler, C. M., & Blumberg, J. B. (1999). Introduction1. The *Journal of nutrition*, *129(3)*, 756S-757S
5. Prajapati, D., Patel, N., Mruthunjaya, K., & Savadi, R. (2009). Antioxidant activity of Actinodaphne hookeri Meissn leaves. J Sci Res, 3, 606-14.
6. Salleh, W. M. N. H. W., & Ahmad, F. (2016). Antioxidant and anti-inflammatory activities of essential oils of Actinodaphne macrophylla and A. pruinosa (Lauraceae). Natural Product Communications, 11(6).
7. Thirumurugan, K., Shihabudeen, M. S., & Hansi, P. D. (2010). Antimicrobial activity and phytochemical analysis of selected Indian folk medicinal plants. steroids, 1(7), 430-34.
8. Uddin, M. N., Alam, T., Islam, M. A., Khan, T. A., Zaman, R. U., Azam, S., ... & Jakaria, M. (2020). Evaluation of carbon tetrachloride fraction of Actinodaphne angustifolia Nees (Lauraceae) leaf extract for antioxidant, cytotoxic, thrombolytic and antidiarrheal properties. Bioscience Reports, 40(6), BSR20201110.