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ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF LEAF EXTRACTS OF *BERBERIS ARISTATA* AND *ABIES WEBBIANA*: THE ETHNOMEDICINAL PLANTS

Sucheta Gautam [1], Neetu Sachan [1], Avijit Mazumder [2], Rupa Mazumder [2] and Dilipkumar Pal [*3]

School of Pharmaceutical Sciences [1], IFTM University Moradabad - 244001, Uttar Pradesh, India
Noida Institute of Engineering and Technology (Pharmacy Institute) [2], Plot no 19, Knowledge Park-2, Greater Noida - 201306, Uttar Pradesh, India.
Department of Pharmaceutical Sciences [3], Guru Ghasidas Vishwavidyalaya (A Central University), Bilaspur - 495009, Chhattisgarh, India.

Keywords:

Abies webbiana, Berberis aristate, Ethnomedicinal plants, Antibacterial and antifungal activities, Minimum inhibitory concentrations, zone of inhibition

Correspondence to Author:
Dilipkumar Pal

Department of Pharmaceutical Sciences, Guru Ghasidas Vishwavidyalaya (A Central University), Bilaspur - 495009, Chhattisgarh, India.

E-mail: drdilip2003@yahoo.co.in

ABSTRACT: This study was carried out to investigate the antibacterial and antifungal potentials of *Abies webbiana* and Berberis aristate Linn. The study aims to assess theantimicrobial activity and determine the zone of inhibition of extracts on some bacterial and fungal strains. The extracts determined the antimicrobial activity using the agar disc diffusion method. The antibacterial and antifungal activities of extracts (5, 25, 50, 100, 250 µg/ml, and so on) of *A. webbiana and B. aristata* were tested against different Gram-positive and Gram-negative bacteria and four fungal strains: *Aspergillus niger*, *Candida albicans,* and *Penicillium notatum* and *Penicillium funiculus*. The zone of inhibition of extracts was compared with that of a standard like ciprofloxacin for antibacterial activity and griseofulvin for antifungal activity. The results showed that the remarkable inhibition of the bacterial as well as fungal growth was shown against the tested organisms. The phytochemical analyses of the plant extracts indicated the presence of saponins, tannins, alkaloids, flavonoids, triterpenoids, steroids, glycosides, anthraquinones, coumarin, saponins, gum, protein, and amino acids. The antimicrobial activity of AWE and BAE may be due to the presence of such secondary metabolites. Hence, these plants may be used to discover bioactive natural products that may lead to the developing of new antimicrobials.

INTRODUCTION: Antibiotics are one of our most important weapons in fighting bacterial infections and have greatly benefited the health-related quality of human life since their introduction. However, over the past few decades, these health benefits are under threat. Many commonly used antibiotics have become less effective against certain illnesses because many produce toxic reactions and the emergence of drug-resistant bacteria.

It is essential to investigate newer drugs with lesser resistance. Drugs derived from natural sources play a significant role in preventing and treating human diseases. In many developing countries, traditional medicine is one of the primary healthcare systems [1-13].

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Herbs are widely exploited in traditional medicine. Their curative potentials are well documented [14.] About 61% of new drugs developed between 1981 and 2002 are based on natural products and have been very successful, especially in infectious disease and cancer [15]. Recent trends, however, show that the discovery rate of active novel chemical entities is declining [16]. Natural products of higher plants may give a new source of antimicrobial agents with possibly novel mechanisms of action [17-18]. The effects of plant extracts on bacteria have been studied by many researchers in different parts of the world [19]. Much work has been done on ethnomedicinal plants in India [20]. Plants are rich in various secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, glycosides, *etc*., which have been found *in-vitro* to have antimicrobial properties [21-22]. Herbal medicines have been known to humans for centuries. Practitioners of traditional medicine [23] have described the therapeutic efficacy of many indigenous plants for several disorders. Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world.

The World Health Organization estimates that plant extracts or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population [24-28]. The harmful microorganisms can be controlled with drugs. These results in the emergence of multiple drug-resistant bacteria and have created alarming clinical situations in treating infections. The pharmacological industries have produced several new antibiotics, and resistance to these drugs by microorganisms has increased gradually. Bacteria have the genetic ability to transmit and acquire resistance to synthetic drugs, which are utilized as therapeutic agents [29].

To expand the spectrum of antibacterial agents from natural resources, *Abies webbiana* has been selected. In the Indian literature, this plant has been described to be useful against skin diseases, liver troubles, and tuberculosis glands, and its use in the treatment of hematemesis, fruits, leukoderma, and diabetes has been suggested. It has been concluded that plant parts could be used as a therapeutic agent in treating hypercholesterolemia partially due to their fiber and mucilage content.

Besides its pharmacological uses, the plant extract is also recommended as a pest and disease control agent in India. *Berberis aristata*is widely used by tribal people to treat various ailments, including ringworm and other fungal skin infections. The leaves are laxative, antiperiodic, depurative, anti-inflammatory and useful in skin diseases, boils, carbuncles, ulcers, intermittent fever, gouty arthritis and rheumatological disorders. *A. webbiana* and *B. aristata* plant organs are known to be an important source of secondary metabolites, they exhibit significant properties that support folkloric use in the treatment of some diseases caused by microbes. Thus, *A. webbiana* and *B. aristata* are well anchored in its traditional uses and now found wide-spread acceptance across the world [29-31].

In the current investigation carried out, a screening of ethanol extracts of *A. webbiana* and *B. aristata* leaves against pathogenic bacteria and fungi is done to detect new sources of antimicrobial agents.

MATERIALS AND METHOD:

Collection of Plant Materials: The fresh and healthy leaves of the plant *A. webbiana and B. aristate* were collected between June and August 2016 from Kalka ji nursey Ranikhet (India). It is commonly known as Daruhaldi. The National botanical research institute, Lucknow, Uttar Pradesh (CIF-RB-3-207) authenticated the plant specimen. Plant parts were collected based on the information provided in the ethnobotanical survey of India. Each specimen/plant material was labelled and numbered and noted with the collection date, locality and medicinal uses recorded.

Preparation of Plant Extract:

Extraction: The leaves were dried in the shade at room temperature. The dried material was coarse powdered and packed in Soxhlet apparatus and extracted with petroleum ether (60-80 °C), ethyl acetate (70-75°C), and ethanol (78°C). The extraction of the *A. webbiana* and *B. aristata* leaves was carried out using known standard procedures [28]. The extracts were filtered using Whatman filter paper (No.1) while hot, concentrated in a vacuum under reduced pressure using a rotary flask evaporator, and dried in a desiccator. The alcoholic extract yields a dark greenish solid residue weighing 5.750 g (23.0% w/w). The ethanol extracts were then kept in sterile bottles, under

refrigerated conditions, until further use. The extract was preserved at 2 to 4°C. These crude alcohol extracts (AWE and BAE, respectively) were used for further investigation for evaluation of antimicrobial properties.

Preliminary Phytochemical Screening: The extracts were subjected to preliminary phytochemical testing to detect the presence of different chemical groups of compounds for the presence of saponins, tannins, alkaloids, flavonoids, triterpenoids, steroids, glycosides, anthraquinones, coumarin, saponins, gum, protein, and amino acids [25-26].

Antimicrobial Activity: The antimicrobial activity of the plant extract was tested *in-vitro* against 21 microorganisms. Stock solutions (1 mg/mL) of both the alcohol extracts of the plants were prepared by dissolving each extract in dimethyl sulfoxide. Calculated volumes of stock solutions were dispensed in series of McCartney bottles previously containing calculated volumes of sterile cooled molten nutrient agar media (40–45°C) to prepare the volume of 30mL each with dilutions of 5, 10, 25, 50, 100,200, 400 and 800µg/mL these sterile nutrient agar media solutions were poured into Petri plates and allowed to solidify.

These plates were kept in the refrigerator at 4°C for 24h to allow the uniform diffusion of the compounds throughout the nutrient agar medium. Before spot inoculation, plates were kept at 37°C for 2 h. One loop full (loop diameter: 3 mm) of an overnight grown peptone-water culture of each microorganism was inoculated and the checkerboard technique marked the location of the inoculation. The spot inoculated plates were incubated at 37°C for 24h, and the minimum inhibitory concentration (MIC, mM) values were obtained [25-37]. The pMIC (−log10MIC) values of both the plant extract *Berberis aristata* (BAE) and *Abies webbiana* (AWE) were calculated. A MIC value of each extract was calculated and compared with that of the standard. Experiments were done in triplicate, and the results were presented as mean values of the three measurements.

Antifungal Activity: The antifungal activities of both the alcoholic extracts of the plants were studied by the disc diffusion assay method.

The pathogenic stains of different fungi given below in **Tables 5-7** were used in the study for performing experiments.

RESULT AND DISCUSSION: Results of antibacterial studies are exhibited in **Tables 1-7.** From the results, it is found that the degree of effectiveness of microbial strain and the percent of inhibition were found to differ in both plant extracts at different concentration values. Both the alcoholic extracts of plants present in the research work showed inhibition against different bacterial and fungal MIC strains ranging from 50-200µg/ml.

In the case of antibacterial activity, the zone of inhibition of ethanol extract of *B. aristate* (BAE) ranged between 6.0 to 16.5 mm. At the same time, the ciprofloxacin showed a zone of inhibition between 16.0 to 20.5 mm. The extract showed lower antibacterial against *Bacillus pumilus* 82, whereas higher antibacterial activity was observed against *Shigella flexneri* Type 6.

The extract showed weak to moderate antibacterial activity against bacteria. The ethanol extract inhibited the growth of *Staphylococcus aureus* ML 267, *Bacillus pumilus* 82 and *Bacillus subtilis* ATCC 6633 at higher concentrations (200 µg/ml). BAE demonstrated maximum antibacterial activity here compared to AWE (**Tables 1-4).**

In the case of antifungal activity, the zone of inhibition of ethanol extract of *A. webbiana* (AWE) ranged between 12.5 to 14.5 mm, while the griseofulvin showed a zone of inhibition between 14.0 to 16.0 mm against fungal. The extract showed lower antifungal against *Candida albicans* ATCC 10231, whereas higher antifungal activity was observed against *Penicillium notatum* ATCC 11625. The extract showed weak to moderate antifungal activity against fungal strain. On the other hand, minimum inhibitory concentration values of BAE at 400 µg/ml were observed towards *Aspergillus niger ATCC 6275* and correspond to the lowest concentration of extract inhibiting fungal growth. The ethanol extract inhibited the growth of *C. albicans ATCC 10231* at higher concentrations (1000 µg/ml). Here, the AWE demonstrated maximum antifungal activity (**Tables 5-7).** The preliminary phytochemical screening found that AWE and BAE possess various phenolic and

flavonoid contents. Although the antibacterial mechanisms of medicinal plants used in this study against various microorganisms were not fully illustrated, we suggest that phenolic and flavonoids may play an important role in their antibacterial activity. We suggest that plant extracts used in this study may become a source for discovering novel antibiotic agents from plant sources.

TABLE 1: DETERMINATION OF MINIMUM INHIBITORY CONCENTRATIONS OF THE SAMPLE CODED BAE

Name of Bacteria	Growth in nutrient agar containing different concentrations of compound BAE in µg/ml								
	0*	5	10	25	50	100	200	400	800
E. coli NCTC 5933	+	+	+	+	+	-	-	-	-
E. coli K88	+	+	+	+	+	-	-	-	-
E. coli NCTC 7360	+	+	+	+	+	-	-	-	-
E. coli LT37	+	+	+	+	+	-	-	-	-
E. coli 872	+	+	+	+	+	-	-	-	-
E. coli ROW 7/12	+	+	+	+	+	-	-	-	-
E. coli 3:37C	+	+	+	+	+	-	-	-	-
E. coli CD/99/1	+	+	+	+	+	-	-	-	-
Salmonella typhi Ty2	+	+	+	+	+	+	+	+	+
Salmonella enterica TD 01	+	+	+	+	+	+	+	+	+
Shigella dysentery 8	+	+	+	+	+	-	-	-	-
Shigella soneii 1	+	+	+	+	-	-	-	-	-
igella boydii D13629	+	+	+	+	-	-	-	-	-
Shigella flexneri Type 6	+	+	+	+	-	-	-	-	-
Staphylococcus aureus ML 267	+	+	+	+	+	+	+	-	-
Bacillus pumilus 82	+	+	+	+	+	+	+	+	+
Bacillus subtilis ATCC 6633	+	+	+	+	+	+	+	+	+
Vibrio cholerae NCTC 4693	+	+	+	+	+	-	-	-	-
Vibrio cholerae NCTC5596	+	+	+	+	+	-	-	-	-
Vibrio cholerae NCTC 10732	+	+	+	+	+	-	-	-	-
Vibrio cholerae NCTC 11501	+	+	+	+	+	-	-	-	-

(0*-control (Sterile DMSO); + growth; - No growth; ± Inhibited growth)

TABLE 2: COMPARISON OF ZONES OF INHIBITION OF BAE WITH THAT OF STANDARD ANTIBACTERIAL AGENT CIPROFLOXACIN AT 200µG/ML

Bacteria	Diameters of zones of inhibition in mm around discs of diameter 6mm at a concentration of 200 µg/ml	
	Compound BAE	Ciprofloxacin
E. coli NCTC 5933	12.5,13,13	16.0, 17.0, 15.0
E. coli K88	12, 12.5,12.5	17.0, 17.0, 17.0
E. coli NCTC 7360	12.5,12.5,12.5	, 16.0, 18.0., 17.0
E. coli LT37	13,12,11	, 16.0, 16.0, 16.0
E. coli 872	12,12.5,12.5	16.0, 15.0, 16.5
E. coli ROW 7/12	13.11, 12.5	16.5, 16.5, 16.5
E. coli 3:37C	12.5,12.5,12	16.5, 17.5, 15.5
E. coli CD/99/1	13, 12.5,12.5	17.0, 16.0, 17.5
Salmonella typhi Ty2	11.5,12.0,12.5	16.0, 16.0, 16.0
Salmonella enterica TD 01	11.5,12.5,12.5	19.0, 19.5, 18.5
Shigella dysentery 8	12.5,11.5,12.5	20.0, 20.0, 20.0
Shigella soneii 1	11.5,11.5,11.5	19.5, 19.5, 19.5
Shigella boydii D13629	12.0,15.0,15.5	20.0, 20.0, 20.0
Shigella flexneri Type 6	15.0,15.5,17.0	20.5, 20.5, 20.5
Staphylococcus aureus ML 267	12.0,12.5,12.0	18.0, 18.0, 18.0
Bacillus pumilus 82	6,6,6	19.0, 18.0, 19.5
Bacillus subtilis ATCC 6633	6.5,6,6	18.0, 18.5, 17.5
Vibrio cholerae NCTC 4693	12,12,12	17.5, 17.5, 17.5
Vibrio cholerae NCTC5596	12.5,12.5,12	18.5, 18.0, 19.5
Vibrio cholerae NCTC 10732	12.5,11.5,10	18.5, 19.0, 19.5
Vibrio cholerae NCTC 11501	11,12.0,11.5	18.5, 18.0, 19.0

TABLE 3: DETERMINATION OF MINIMUM INHIBITORY CONCENTRATIONS OF THE SAMPLE CODED AWE

Name of Bacteria	Growth in nutrient agar containing different concentrations of compound AWE in µg/ml								
	0*	5	10	25	50	100	200	400	800
E. coli NCTC 5933	+	+	+	+	-	-	-	-	-
E. coli K88	+	+	+	+	-	-	-	-	-
E. coli NCTC 7360	+	+	+	+	-	-	-	-	-
E. coli LT37	+	+	+	+	-	-	-	-	-
E. coli 872	+	+	+	+	-	-	-	-	-
E. coli ROW 7/12	+	+	+	+	-	-	-	-	-
E. coli 3:37C	+	+	+	+	-	-	-	-	-
E. coli CD/99/1	+	+	+	+	-	-	-	-	-
Salmonella typhi Ty2	+	+	+	+	-	-	-	-	-
Salmonella enterica TD 01	+	+	+	+	-	-	-	-	-
Shigella dysentery 8	+	+	+	+	-	-	-	-	-
Shigella soneii 1	+	+	-	-	-	-	-	-	-
Shigella boydii D13629	+	+	-	-	-	-	-	-	-
Shigella flexneri Type 6	+	+	+	+	-	-	-	-	-
Staphylococcus aureus ML 267	+	+	+	+	+	+	+	-	-
Bacillus pumilus 82	+	+	+	+	+	+	+	-	-
Bacillus subtilis ATCC 6633	+	+	+	+	+	+	+	-	-
Vibrio cholerae NCTC 4693	+	+	+	-	-	-	-	-	-
Vibrio cholerae NCTC5596	+	+	+	-	-	-	-	-	-
Vibrio cholerae NCTC 10732	+	+	+	-	-	-	-	-	-
Vibrio cholerae NCTC 11501	+	+	+	-	-	-	-	-	-

(0*-control (Sterile DMSO); + growth; - No growth; ± Inhibited growth)

TABLE 4: COMPARISON OF ZONES OF INHIBITION OF AWE WITH THAT OF STANDARD ANTIBACTERIAL AGENT CIPROFLOXACIN AT 200µG/ML

Bacteria	Diameters of zones of inhibition in mm around discs of diameter 6mm at a concentration of 200µg/ml	
	Compound AWE	Ciprofloxacin
E. coli K99	13,12,12	16.0, 17.0, 15.0
E. coli K88	12.5,12.5,12.5	17.0, 17.0, 17.0
E. coli 306	13.0,13.5,12.5	16.0, 18.0., 17.0
E. coli LT37	12.5,12.5,12.5	16.0, 16.0, 16.0
E. coli 872	13,12.5,12.5	16.0, 15.0, 16.5
E.coli ROW 7/12	11,12,13	16.5, 16.5, 16.5
E.coli 3:37C	12,11,11.5	16.5, 17.5, 15.5
E.coli CD/99/1	12,12.5,12.5	17.0, 16.0, 17.5
Salmonella typhi Ty2	12.5,14.5,14	16.0, 16.0, 16.0
Salmonella enterica TD 01	13.0,14.0,15.0	19.0, 19.5, 18.5
Shigella dysentery 8	14.0,13.0,14.0	20.0, 20.0, 20.0
Shigella soneii 1	15.0,16.5,16.0	19.5, 19.5, 19.5
Shigella boydii D13629	16.0,16.0,15.5	20.0, 20.0, 20.0
Shigella flexneri Type 6	15.5,16.5,14.5	20.5, 20.5, 20.5
Staphylococcus aureus ML 267	14.0,14.5,15.5	18.0, 18.0, 18.0
Bacillus pumilus 82	6.0,6.0,6.0	19.0, 18.0, 19.5
Bacillus subtilis ATCC 6633	6.0,6.0,6.0	18.0, 18.5, 17.5
Vibrio cholerae 1313	13,13.5,13.5	17.5, 17.5, 17.5
Vibrio cholerae 293	14.0,15.0,15.0	18.5, 18.0, 19.5
Vibrio cholerae 1315	14.5,14.5,15.5	18.5, 19.0, 19.5
Vibrio cholerae 85	14,14,14	18.5, 18.0, 19.0

TABLE 5: DETERMINATION OF MINIMUM INHIBITORY CONCENTRATIONS OF THE SAMPLE CODED BAE

Name of Fungi	Growth in Saborauds Dextrose Agar media containing different concentrations of compound coded BAE (µg/ml)								
	0*	50	100	200	400	800	1000	1500	2000
Candida albicans ATCC 10231	+	+	+	+	+	+	-	-	-
Aspergillus niger ATCC 6275	+	+	+	+	-	-	-	-	-
Penicillium notatum ATCC 11625	+	+	+	+	+	-	-	-	-
Penicillium funiculosum NCTC 287	+	+	+	+	+	-	-	-	-

TABLE 6: DETERMINATION OF MINIMUM INHIBITORY CONCENTRATIONS OF THE SAMPLE CODED AWE

Name of Fungi	Growth in Saborauds Dextrose Agar media containing different concentrations of compound coded AWE(µg/ml)								
	0*	50	100	200	400	800	1000	1500	2000
Candida albicans ATCC 10231	+	+	+	+	+	+	-	-	-
Aspergillus niger ATCC 6275	+	+	+	+	+	-	-	-	-
Penicillium notatum ATCC 11625	+	+	+	+	+	-	-	-	-
Penicillium funiculosum NCTC 287	+	+	+	+	+	-	-	-	-

TABLE 7: COMPARISON OF ZONES OF INHIBITION OF VARIOUS SAMPLES AT 2000µg/ml

Name of the Fungi	Diameters of zones of inhibition in mm around discs of diameter 6mm at a concentration of 2000 µg/ml of coded drug in Saborauds Dextrose Agar media		
	BAE	AWE	Griseofulvin (Ref)
Candida albicans ATCC 10231	12	12.5	16.0
Aspergillus niger ATCC 6275	13	14	15.0
Penicillium notatum ATCC 11625	13.5	14.5	15.0
Penicillium funiculosum NCTC 287	13.0	13.5	14.0

CONCLUSION: It is concluded that ethanol extract of *B. aristate* demonstrated maximum antibacterial activity compared to ethanol extract of *A. webbiana*. On the other hand, ethanol extract of *A. webbiana* Showed maximum antifungal activity compared to the ethanol extract of *B. aristate*. From the preliminary phytochemical screening, it was found that ethanol extract of *A. webbiana* and *B. aristate* possessed various phenolic and flavonoids contents. It is suggested that phenolic and flavonoids played an important role in their antibacterial activity.

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