Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2016; 5(2): 265-269
Received: 26-01-2016
Accepted: 27-02-2016

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Evaluation of pharmaceutical properties on microbial activities of some important medicinal orchids of Bangladesh

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Abstract
Three species of medicinal Orchid plants namely; *Acampe papillosa, Aerides odoratum and Pholidota pallida* were extracted with ethanol, chloroform, petroleum ether and methanolic extract assayed for antimicrobial activity against five bacterial strains namely: *Bacillus subtilis, Staphylococcus aureus, Salmonella typhi, Shigella dysenteriae* and INABA-ET *(Vibrio).* Aqueous extracts of the plant also screened for their antifungal activity against six phytopathogenic fungi such as *Alternaria alternata* (Fr.) Kedissler., *Botryodiplodia theobromae* Pat., *Colletotrichum corchori* Ikata (Yoshida), *Curvularia lunata* (Wakker) Boedijin, *Fusarium equiseti* (Corda) Sacc., *Macrophomina phaseolina* (Maubl) Ashby. Here, extracts showed varied degree of activity against the bacterial and fungal strains. Three, extracts showed broad-spectrum antibacterial activity against all the test organisms and among them *Aerides odorata* Lour. And *Pholidota pallida* Lindl. Showed prominent antibacterial activity. The highest inhibition was recorded with *Aerides odorata* Lour. And *Pholidota pallida* Lindl. Against *Salmonella typhi* (37.5mm) and *Bacillus subtilis* showed wide reange of antifungal activity with remarkable inhibition against all the tested phytopathogenic fungi. The highest antifungal activity was recorded with the plant extract of *Aerides dratum* Lour. Against *Colletotrichum corchori* (57.14%) followed by *Alternaria alternata* (57.14%). The present study indicates the antibacterial and antifungal properties in the crude extract of three orchid plants. So, the plants can be used as a novel antimicrobial agent.

Keywords: Antifungal activity**,** medicinal orchids, antimicrobial agent

1. Introduction
Orchids have been very closely associated with human being since ancient times. It has not only the horticultural value but also good medicinal value. Many orchids play a significant role in traditional system of medicine because they are rich in alkaloids, flavonoids, glycosides, carbohydrates and other photochemical contents [1]. Fifty three orchids, species belonging to 34 genera have various medicinal properties [2]. *Acampe papillosa* (Lindl.) Lindl. Roots are used in rheumatism [3] to cure rheumatism, sciatica, neuralgia, syphilis, uterine diseases and as tonic. *Aerides odorata* Lour; Ground fruit was used in Indochina to treat wounds [4]. Extracted juice from the pseudobulbs of *Bulbophyllum* sp. are use for restoration of adolescence and as tonic [5] [3], *Pholidota pallida* Lindl. Leaves and roots were used in bone fracture [5]. The Himalayan squirrel likes to eat the inflorescence [6]. In Indochina this plant is used to bathe depilated infants and to treat menstrual irregularity and also to treat burns and sores [7]. Microorganisms have developed resistant to many antibiotics and this has created immense clinical problems in the treatment of infectious diseases [8]. This situation forced scientists to search for new antimicrobial substance from various sources, such as medicinal plants. Various plant species have been serving as the best natural source of drugs and medicines from the beginning of civilization. Among the different plant derived, secondary metabolites proved to be the most important group of compounds that showed wide range of antibacterial and antifungal agents since none of the available drugs is free from adverse effects and limitation. But medicinal plants possess various remedial properties with worthless materials and it is important to separate those materials from the good ones. Now-a-days, the natural products have been interesting and important sources of biologically active antimicrobial substances and the major sources of new materials which are still left undiscovered. It is evident from various reports that orchids have ingredient to cure various diseases. The present work was undertaken to investigate the pharmaceutical properties on antibacterial and antifungal potentiality of these orchid species which are indigenous to Bangladesh.

2. Material and Methods

2.1 Extraction of plant Materials

The plant samples [*Acampe papillosa* (Lindl.) Lindl, *Aerides odoratum* Lour. And *Pholidota pallida* Lindl.] Were collected from Cox's Bazar Bangladesh. Firstly, the collected samples were properly identified and cleaned. The fresh samples were always used for extraction. The plant parts were cut into small pieces (1-2 cm) and placed in a sterilized conical flask. After word, sterilized distilled water was added with a ratio of 1:2 (w/v) and kept it for twenty four hours. After 24 hours, the water soaked samples were crushed with a pastel in mortar. Then several layer of cheese cloth and finally filtered with Whateman NO. 1 filter paper. Then the filtrates were sterilized in a autoclave at 121°C and 15 lbs. pressure for 15 minutes. In a similar procedure as mentioned above, 95% alcohol, chloroform and petroleum ether (40-60°C) extracts were made which were used without sterilization in a autoclave.

2.2 Test bacteria

In this study five human pathogenic bacteria *Bacillus subtilis* BTCC 17, *Staphylococcus aureus* ATCC 6538, *Salmonella typhi* AE 14612, *Shigella dysenteriae* AE 14396, INABA-ET *(Vibrio)* AE 14748 were used as test organisms. Bacterial test organisms were collected from Department of Microbiology, University of Chittagong, Bangladesh.

2.3 Antibacterial test

Sensitivity spectrum analysis was done by paper disc method of Bauer *et al.* [9]. Paper disc of 6 mm in diameter and Petri plate of 70 cm in diameter were used throughout the experiment. These paper discs were sterilized in autoclave and dried at 60 ^{0}C in oven. Then the discs were soaked with plant extracts (Petroleum ether; Alcohol, Chloroform and Water) and alkaloid solution separately for antimicrobial analysis. After solidification of Nutrient agar (NA) medium (pour plate), the discs with extracts water; chloroform; petroleum ether (40-60 ^{0}C) and alcohol extract of plant separately, and after removing the excess extract discs were placed at the centre of the medium. A control plate (with organic solvent or water) was also maintained in each case. In case of antimicrobial activity of alkaloid the disc was soaked with 25 µl of crude alkaloid solution (50% alcoholic suspension) and then disc were placed on the centre of the solidified medium. A control plate was also maintained in each experiment. The plates firstly were kept for 24 hours at low temperature (4 ^{0}C) and the test compound diffused from disc to the surrounding medium by this time. The plates were then incubated at 35±2 ^{0}C for growth of test organisms and were observed at 24 hours interval for two days. The activity expressed in terms of inhibition zone diameter in mm. Each experiment was repeated thrice.

2.4 Test Fungi

Six phytopathogenic fungi *Alternaria alternate* (Fr.) Kedissler, *Botryodiplodia theobromae* Pat., *Colletotrichum corchori* Ikata (Yoshida), *Curvularia lunata* (Wakker) Boedijin, *Fusarium equiseti* (Corda) Sacc, *Macrophomina phaseolina* (Maubl) Ashby. Were used as test organisms. Fungi test organisms were collected from, Department of Microbiology, University of Chittagong, Bangladesh.

2.5 Plant pathogenic fungi test

Radial growth inhibition test according to Kapoor *et al.* [10] was followed in the present study. The plant extract (aqueous) and crude alkaloid were tested following food poison technique of Grover and Moore [11]. Plant extract (aqueous) were mixed with potato Dextrose Agar (PDA) medium to have 50% concentration of crude extract. After autoclaving at 121 ^{0}C and 15 Ibs pressure for 15 minutes. The medium as poured in sterilized Petri plate and after solidification the fungal inoculums (10 mm mycelial block) was placed on the centre of the Petri plate. A control set of fungal plates was also prepared (without plant extract). 20 ml PDA medium were mixed uniformly with 25 µl of crude alkaloid. From 5 days old fungal culture, inoculums (10 mm mycelial block) were made by using sterilized cork borer and a long glass rod (for each fungus) and placed on the centre of solidified plate that were made following the food poison technique of Grover and Moore [11]. The experiment was repeated thrice. Radial growth of fungal colony was measured in mm. after 5 days of incubation at 25±2 ^{0}C. A control set was maintained in each experiment using only PDA as growth medium. The percentage inhibition of mycelial growth of test fungi was calculated as follows:

$$I = \frac{C-T}{C} \times 100$$

Here, I = Percentage of inhibition, C = Diameter of fungal colony in control and T = Diameter of fungal colony in treatment.

3. Results and Discussion

3.1 Antibacterial test

In this study, Ethanol, chloroform, petroleum ether and methanol extracts from whole plant of *Acampe papillosa* were tested against five human pathogenic bacteria to determine the antimicrobial potentiality of this plant and compared with antimicrobial agent ampicillin (Table 1). It was found that the petroleum ether extract was not active against all the five bacteria tested. Methanolic extract showed maximum zone of inhibition (25 mm) against *Salmonella typhi* (Fig. 1a). On the other hand, chloroform extract and ethanol extract showed moderate antimicrobial activity against different bacterial strains.

Table 1: Antibacterial activities of different extracts from the whole plant of *Acampe papillosa* against pathogenic Bacteria.

Name of Bacteria	Gram +/-	Diameter of inhibition zone in mm				
		Ampicillin 25 µg/disc	Leaf extract (soaked)*			
			Ethanol	Chloroform	Pet-ether	Methanol
1. *Bacillus subtilis*	+	21	15	23	-	-
2. *Staphylococcus aureus*	+	20	-	-	-	-
3. *Shigella dysenteriae*	-	30	10	-	-	-
4. *Salmonella typhi*	-	24	-	-	-	25**
5. INABA-ET (*Vibrio*)	-	17	15	-	-	-

Note: **After removing the excess leaf extract from the paper disc. ** Markable zone of inhibition. (-) Minus sign indicates no zone of inhibition.*

Five human pathogenic bacteria were used to evaluate the antibacterial activities of ethanolic, chloroform, petroleum ether and methanolic extracts of *Aerides odoratum* leaves and compared with antibacterial agent ampicillin (Table 2). It was found that all the extracts were active against most of the tested bacteria. The maximum zone of inhibition (37.5 mm) was recorded with ethanol extract against *Salmonella typhi* (Fig.1b). Methanolic extract of this plant also showed remarkable zone of inhibition 26 mm against *Salmonella typhi.* On the other hand, petroleum ether extract of this plant also showed remarkable zone of inhibition 27.5 mm, 25 mm and 26 mm, against *Bacillus subtilis,* INABA-ET (*vibrio*) and *Salmonella typhi,* respectively.

Table 2: Antibacterial activities of different extracts from the leaves of Aerides *odoratum* against pathogenic Bacteria.

Name of Bacteria	Gram +/-	Diameter of inhibition zone in mm				
		Ampicillin 25 µg/disc	Leaf extract (soaked)*			
			Ethanol	Chloroform	Pet-ether	Methanol
1. *Bacillus subtilis*	+	21	16	17	27.5	-
2. *Staphylococcus aureus*	+	20	-	16	32	-
3. *Shigella dysenteriae*	-	30	25	10.5	10	8
4. *Salmonella typhi*	-	24	37.5**	-	12	26
5. INABA-ET (*Vibrio*)	-	17	-	10	25	15

Note: **After removing the excess leaf extract from the paper disc. ** Markable zone of inhibition. (-) Minus sign indicates no zone of inhibition.*

In Table 3 five human pathogenic bacteria were used to test the potentiality of antibacterial activities of ethanolic, chloroform, petroleum ether and methanolic extracts. The extracts were extracted from whole plant of *Pholidota pallida* and compared with antibacterial agent ampicillin. It was found that all the extracts were active against most of the tested bacteria. The maximum zone of inhibition (23 mm) was recorded with petroleum ether extract against *Bacillus subtilis.* Chloroform extract of this plant also showed remarkable zone of inhibition against INABA-ET (*Vibrio*) and *Salmonella typhi* 20 mm and 25 mm respectively. In this Table, it was observed that Pet-ether extract was active against *Bacillus subtilis* but no inhibition zone was found against other four types of bacteria.

Table 3: Antibacterial activities of different extracts from the whole plant of *Pholidota pallida* against pathogenic Bacteria.

Name of Bacteria	Gram +/-	Diameter of inhibition zone in mm				
		Ampicillin 25 µg/disc	Leaf extract (soaked)*			
			Ethanol	Chloroform	Pet-ether	Methanol
1. *Bacillus subtilis*	+	21	-	15	23**	-
2. *Staphylococcus aureus*	+	20	-	22	-	-
3. *Shigella dysenteriae*	-	30	20	-	-	-
4. *Salmonella typhi*	-	24	15	25	-	-
5. INABA-ET (*Vibrio*)	-	17	-	20	-	15

Note: **After removing the excess leaf extract from the paper disc. ** Markable zone of inhibition. (-) Minus sign indicates no zone of inhibition.*

3.2 Antifungal activity

In Table 4 six phytopathogenic fungi were tested against water extract of whole plant of *Acampa papillosa,* leaves of *Aerides odoratum* and whole plant of *Pholidota pallida* compared with that of antifungal agent nystain. In this Table, it was found that the water extract was tested and found that it was inhibit the mycelial growth remarkably in case of all phytopathogenic fungi. *Colletotrichum corchori,* 45.50% (Fig.2a), *Alternaria alternata*, 55% (Fig.2b) and *Fusarium equiseti*, 50% showed comparatively broad-spectrum inhibition of mycelial growth.

Table 4: Antifungal activities of water extracts isolated from the leaves of *Acampe papillosa against* six phytopathogenic fungi.

Name of Fungi	% of growth inhibition	
	Leaf extract/plate*	Nystatin plate** 100 µg (dw)/ml PDA
1. *Alternaria alternata*	55.00	51.55
2. *Curvularia lunata*	60.00	75.00
3. *Colletotrichum corchori*	45.50	40.51
4. *Fusarium equiseti*	50.00	44.70
5. *Macrophomina phaseolina*	33.30	71.78
6. *Botryodiplodia theobromae*	50.00	70.05

Note: **Water extract: PDA = 1:1 ** Standard antifungal antibiotic.*

The mycelial growth of all phytopathogenic fungi was inhibited by the water extract of *Aerides odoratum* plant (Table-5). The maximum inhibition of mycelial growth was recorded against 57.17% for *Alternaria alternate,* 57.14% for *Colletotrichum corchori* and 64.28% for *Fusarium equiseti*.

Table 5: Antifungal activities of water extracts isolated from the leaves of *Aerides odoratum* against six phytopathogenic fungi.

Name of Fungi	% of growth inhibition	
	Leaf extract/plate*	Nystatin plate** 100 µg (dw)/ml PDA
1. *Alternaria alternata*	57.14	51.55
2. *Curvularia lunata*	71.42	75.00
3. *Colletotrichum corchori*	57.14	40.51
4. *Fusarium equiseti*	64.28	44.70
5. *Macrophomina phaseolina*	64.28	71.78
6. *Botryodiplodia theobromae*	57.14	70.05

Note: **Water extract: PDA = 1:1 ** Standard antifungal antibiotic.*

It was observed that water extract of this plant inhibited the mycelial growth remarkably in case of all phytopathogenic fungi. *Alternaria alternata*, (57.14%), *Colletotrichum*

corchori, 66.67% (Fig. 2c) and *Fusarium equiseti*, 50% showed broad spectrum inhibition of mycelial growth (Table 6).

Table 6: Antifungal activities of water extracts isolatrd from the leaves of *Pholidota pallida* against six phytopathogenic fungi.

Name of Fungi	% of growth inhibition	
	Leaf extract/plate*	Nystatin plate** 100 µg (dw)/ml PDA
1. *Alternaria alternata*	57.14	51.55
2. *Curvularia lunata*	66.67	75.00
3. *Colletotrichum corchori*	66.67	40.51
4. *Fusarium equiseti*	50.00	44.70
5. *Macrophomina phaseolina*	57.14	71.78
6. *Botryodiplodia theobromae*	42.86	70.05

Note: **Water extract: PDA = 1:1 ** Standard antifungal antibiotic.*

The antibacterial and antifungal activities of ethanol, chloroform, petroleum ether, methanol extracts and water extract of whole plant or leaves of *Acampe papillosa* Lindal, *Aerides odoratum* Lour, *Pholidota pallida* Lindal. Were tested against five human pathogenic bacteria and six phytopathogenic fungi and compared with antibacterial agent Ampicillin and antifungal agent nystatin. Similar antimicrobial activities had been found which were reported by Ghanaksh *et al*. [12], Abhishek *et al*. [13]. The *Aerides odoratum* plant extracts were active against most of the tested bacteria. The maximum zone of inhibition was recorded 37.5 mm with ethanol extract against *Salmonella typhi*, methanolic extract of this plant also showed remarkable zone of inhibition 26 mm and petroleum ether extract of this plant also showed remarkable zone of inhibition 27.5 mm, 25 mm and 26 mm against *Bacillus subtilis*, INABA-ET (*Vibrio*) and *Salmonella typhi* respectively (Table 2). Similar antibacterial activities of plant extracts from other plants have been reported previously by a number of investigators Hassan [14], Sokomba *et al*. [15], Ahmed *et al*. [16], which is an agreement with present research findings. *Aerides odoratum* water extract also inhibited mycelial growth of all phytopathogenic fungi. The maximum inhibition of mycelial growth 64.28% was recorded against *Fusarium equiseti* (Table 5). The *Acampe papillosa* Lindal, plant extracts were active against all most all of the test bacteria. *Bacillus subtilis* showed the maximum zone of inhibition with petroleum ether extract. However, petroleum ether extract of *Pholidota pallida* also showed markable zone of inhibition 23 mm. The water extracts of *Acampe papillosa* was inhibited the mycelial growth of all test phytopathogenic fungi. The maximum mycelial growth inhibition was 45.50% and 50% against *Colletotrichum corchori* and *Fusarium equiseti* respectively (Table 4). *Alternaria alternata* also showed 55% inhibition zone of mycelial growth. The maximum zone of inhibition was observed in chloroform extract of whole plant *Pholidota pallida* 20 mm against INABA-ET (*Vibrio*) in Table 3. Extract of petroleum ether of this plant also showed markable zone of inhibition 23 mm against *Bacillus subtilis*. 20 and 22 mm zone of inhibition was found against INABA-ET (*Vibrio*) and *Staphylococcus aureus*, respectively (Table 3). The water extract of *Pholidota pallida* was also found to inhibit the mycelial growth remarkably in case of all phytopathogenic fungi. The zone of inhibition was recorded 66.67%, 57.14% and 50% in *Colletotrichum corchori*, *Alternaria alternata* and *Fusarium equiseti*, respectively, which was broad-spectrum inhibition of mycelial growth. Similar antimicrobial activities of extracts from other plants have been found in many previous reports on Miah *et al*. [17], Rahman *et al*. [18], Ramesh *et al*. [19], which collaborate with present research outcome.

It can be concluded from the overall observation of above discussions that extract of orchid plants have strong antimicrobial properties. Therefore, orchid can be used as raw materials for pharmaceutical industries, which will open new dimension in the field of commercialization of orchids as source of medicinally active ingredients.

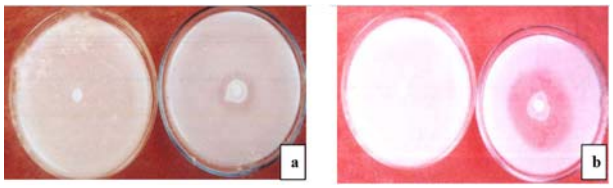

Fig 1: Zone of inhibition produced by the extracts from (a) Leaves of *Acampe papillosa* against *Salmonella typhi* (b) leaves of *Aerides odoratum* against *Salmonella typhi*

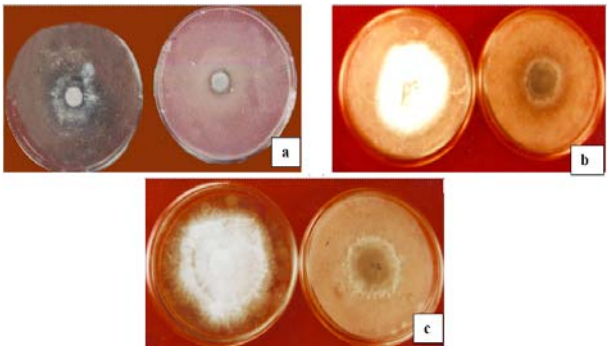

Fig 2: Percentage of growth inhibition produced by the whole plant extracts of (a) *Pholidota pallida* against *Colletotrichum corchori* (b) *Pholidota pallida* against *Fusarium equiseti* (c) *Aerides odoratum* against *Colletotrichum corchori*

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