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EVALUATION OF ANTIMICROBIAL PROPERTIES OF TERRESTRIAL ORCHIDS (COLLECTED FROM NORTHERN HIMALAYAS) AGAINST CERTAIN HUMAN PATHOGENS

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Abstract: The four main varieties of orchids, collected from northern Himalayas (Tara devi and Chhrabra forests, Shimla, HP) were evaluated for their antimicrobial activity against human pathogenic bacteria. The ethanol and methanol extracts of *Cypripedium cordigerum* and *Malaxis acuminata* were found to be highly active against both *P.aeruginosa* and *S.aureus* with minimal microbial static concentration (MIC) in the range of 100mg/ml. These plants particularly demonstrated antimicrobial properties against Gram negative bacterial strains, which are responsible for severe opportunistic bacterial infection and are resistant to hospitalized infections. These orchid species may thus, be considered important tools in antibacterial strategies. It can be concluded that orchid family represent an untapped source of potentially useful antibacterial products and are worthy of further study.

Key Words: Antimicrobial activity, Extract, Inhibition, Orchid, Solvent.

INTRODUCTION

Orchidaceae is regarded as one of the largest, most diverse and distinctive families in the flowering plant kingdom with estimates of about 17,000 to 35,000 species in the world [1]. In this research, we have studied various species of orchids that were harvested from the northern Himalayas (Shimla, HP) such as the forests of Taradevi (1800m, 7kms from Shimla) and Chhrabra (2300m, 13 kms from Shimla). Terrestrial orchids usually grow on the ground where sufficient moisture and shade is available and most of them generally appear during the rainy season [2]. The orchid species collected were identified as *Habenaria intermedia*, *Cypripedium cordigerum*, *Malaxis acuminata* and *Satyrium nepalense*. These plants were identified on the basis of their morphological and physiological characteristics. In Karnataka, it is distributed in Belgaum, Kodagu, Chickmagalur, Mysore and Uttarakannada areas. Flowering and fruiting in such plants occurs from the month of April to July [3]. But the orchid species so collected are rarely found in rainy season.

They exist in almost all ecological conditions except the marine environment and the extreme cold climates. Environmental conditions associated with altitude exert a great influence on orchid species composition and their distribution [4]. Their diversity increases towards the tropic. The orchids may be terrestrial, epiphytic, lithophytic and saprophytic in habitat [5]. The epiphytic orchids are the predominant orchid species among others, constituting about 73% of the family. The past record of rapid, widespread emergence of resistance to newly introduced antimicrobial agents indicates a short life expectancy for the new families of antimicrobial agents [6]. For this reason, researchers are increasingly turning their attention to herbal products, looking for new leads to develop better drugs against MDR microbe strains [7]. Orchids, particularly the species *Dendrobium officinale*, have been used as medicinal herbs for centuries. New research has discovered many new applications of the different orchid species, and today there are at least 50 orchid species used especially in traditional Chinese medicine formulas. The use for dried orchids ranges from immune system build-up, to cancer treatment, eye-sight improvement and regaining strength after healing (for healers). The parts of the orchids used most frequently are the stems and bulbs, as they are designed to keep the plant alive during dry season, and hence are rich in nutritive substances [8].

The previous studies on orchids have shown them to be rich in certain biochemicals as carbohydrates, flavonoids, alkaloids, glycosides and other phytochemical contents which have great importance in medicinal field. The orchids were first put to medicinal use by the Chinese as herbal medicine [9]. A wide range of chemical compounds including alkaloids, bibenzyl derivatives, flavonoids, phenanthrenes and terpenoids have been recently isolated from the orchid species. Extracts and metabolites of these plants, particularly those from flowers and leaves, possess useful pharmacological activities. Particular attention has been given to diuretic, antirheumatic, anti-inflammatory, anti-carcinogenic, hypoglycemic activity, antimicrobial,

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anticonvulsive, relaxation, neuroprotective, and antivirus activities. A large number of orchids have been empirically used for treatment of different diseases, thus, several studies have been undertaken to provide scientific proof to justify the medicinal use of various plants in the treatment of diseases [10].

In the present study these plants were checked for their antibacterial properties against some pathogenic microbes in humans. Different types of extracts using different parts of the plant were prepared which were then subjected to sensitivity analyses against the bacterial agent. Certain constituents of orchids were found to be suggestive of biological activity [11].

MATERIALS AND METHODS

Collection of Plants

Four different types of orchid plants were collected from the Shimla Hills at Tara Devi (1800m, 7kms before Shimla) and Chhrabra (2300m, 13 kms from Shimla). The plants were identified as *Habenaria intermedia, Cypripedium cordigerum, Malaxis acuminata* and *Satyrium nepalense*.

Preparation of Plant Extracts

The leaves, roots and tubers of the plants were preferably shade-dried to prevent the loss of active phyto-constituents and then ground to fine powder using a mechanical grinder. The plant extracts were prepared by cold maceration method involving the solvents of differing polarity. About 1g of the dried plant powder was soaked into each of the solvents viz., distilled (autoclaved) water, ethanol, methanol and chloroform, depending upon the polarity indices of each, in the same sequence and kept for 72 hours. The extracts were filtered through Whatman filter paper No. 3, concentrated to dryness, covered and stored at room temperature for further studies. Calculated amount of the extracts were dissolved both in dimethyl sulphoxide (DMSO) and the individual solvents under aseptic conditions to prepare the desired dilutions. After the evaporation of the solvent, the material stuck to the bottom of the beaker was scraped off and collected.

Susceptibility Test by Agar Well Diffusion Method

Preparation of Müeller-Hinton Agar: Muller-Hinton agar was prepared from a commonly available base according to the manufacturer's instructions. After autoclaving, it was allowed to cool in a 45-50°C water bath. The freshly prepared medium was poured into Petri dishes. The agar medium was allowed to cool at room temperature and stored in a refrigerator (2-8°C).

Preparation of Test Microorganisms: The pathogenic microbial strains namely *Pseudomonas aeruginosa* (MTCC 4676) and *Staphylococcus aureus* (MTCC 7405) were obtained from Institute of Microbial Technology, Chandigarh. All the microbial strains were maintained on nutrient agar plate and transferred to the nutrient broth at the time of checking the activity and stored at 37°C for 4-8hours.

Preparation of Antimicrobial Extracts: The dried extracts were weighed 100mg and dissolved in 100ml of the same solvent in which they were prepared.

Inoculation of Test Plates: After the incubation of the broth cultures, a sterile cotton swab was dipped into the suspension. The dried surface of a Muller-Hinton agar plate was inoculated by streaking swab over the entire sterile agar surface. This procedure was repeated by streaking two or more times.

Pouring Extracts onto Inoculated Agar Plates: The wells were then cut with 6mm diameter using a cork borer, and about 30µl of the crude extracts were poured into each well. Not more than four wells were cut into each plate. The plates were placed in an incubator and set to 37°C within 15 minutes after the pouring the antimicrobial extract.

Analyses of Zone Size and Interpreting Results: After 16 to 18 hours of incubation, each plate was examined. The diameters of zones of complete inhibition (as analyzed by the unaided eye) were measured, including the diameter of the well. Zones were measured to the nearest area, using sliding calipers, which was held on the back of the inverted petri plate. The inhibition zone is represented in Fig. 1 (A & B)

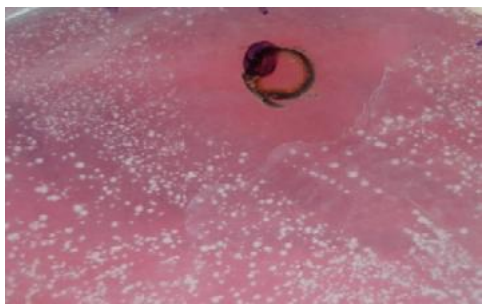

Fig.1: (A) Formation of Inhibition Zones by the Well Diffusion Method. The extract diffused out radially and thus inhibited the growth of bacteria.

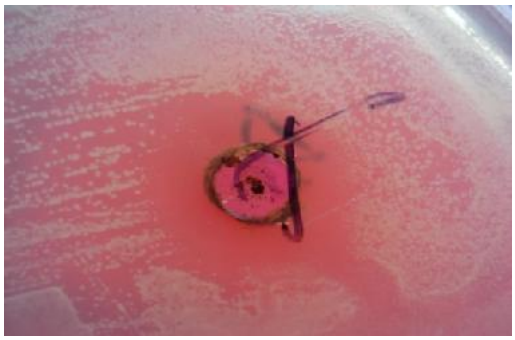

Fig.1: (B) Formation of Inhibition Zones by the Well Diffusion Method. The extract diffused out radially and thus inhibited the growth of bacteria.

RESULTS

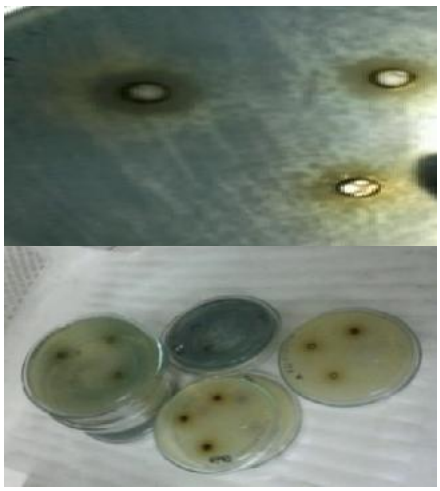

Fig.2: Analysis of Cultured Plates with Both the Cultures i.e. *Pseudomonas* and *Staphylococcus.*

In this susceptibility test, the concentration of the extract used was 100mg/ml. This sensitivity test yielded the zones of inhibition of varying sizes (recorded by measuring the diameter of the zone) shown in table 1 & 2. Colistin was used as a positive control on *Staphylococcus aureus* and Doripenam on the *Pseudomonas aeruginosa* as shown in Fig.2. The solvent was referred to as the negative control. The tests were carried out in the following sequence.

Test 1. Susceptibility testing of extracts of *Habenaria intermedia* (leaves) with various solvents against *Staphylococcus aureus* and *Pseudomonas aeruginosa.*

Test 2. Susceptibility testing of extracts of *Malaxis acuminata* with various solvents against *Staphylococcus aureus* and *Pseudomonas aeruginosa*

Test 3. Susceptibility testing of extracts of *Satyrium nepalense* with various solvents against *Staphylococcus aureus* and *Pseudomonas aeruginosa*

Test 4. Susceptibility testing of extracts of *Cypripedium cordigerum* with various solvents against *Staphylococcus aureus* and *Pseudomonas aeruginosa*

Test 5. Susceptibility testing of extracts of *Habenaria intermedia* (tubers) with various solvents against *Staphylococcus aureus* and *Pseudomonas aeruginosa*

Table 1: Zone of Inhibition of Test Extract against *Staphylococcus aureus*

Plant extracts	Diameter of zone of inhibition Test 1 (mm)	Diameter of zone of inhibition Test 2 (mm)	Diameter of zone of inhibition Test 3 (mm)	Diameter of zone of inhibition Test 4 (mm)	Diameter of zone of inhibition Test 5 (mm)
Ethanol	6	12	6	15	11
Methanol	9	14	6.5	16	8
Chloroform	6	8	6	8	12
Aqueous	6	9	6.5	7	6
Positive Control	16	13	15	13	15
Negative Control	6	6	6	6	6

Table 2: Zone of Inhibition of Test Extract against *Pseudomonas aeruginosa*

Plant extracts	Diameter of zone of inhibition Test 1 (mm)	Diameter of zone of inhibition Test 2 (mm)	Diameter of zone of inhibition Test 3 (mm)	Diameter of zone of inhibition Test 4 (mm)	Diameter of zone of inhibition Test 5 (mm)
Ethanol	8	11	6.5	12	10.5
Methanol	8	13	6	15	9
Chloroform	9.5	7	7	8	14
Aqueous	7	7	6	7	6
Positive Control	15	14	13	14	14
Negative Control	6	6	6	6	6

CONCLUSION

It can thus be inferred from the present study that orchids are endowed with a good amount of antimicrobial properties. The possession of such properties by the orchids can be attributed to the presence of certain biochemicals and phytochemical contents. The ethanolic and methanolic extracts of specifically the tuber portion of the plant was found to be highly active against the test bacteria. The maximum activity was shown by *Malaxis acuminata* and the least by *Satyrium nepalense.* Thus, owing to the great potential exhibited by orchid species against pathogenic bacteria, they can be the subject of study for various medicinal purposes.

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