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Research Article

ANTIMICROBIAL EFFICACY OF ORCHID EXTRACTS AS POTENTIAL INHIBITORS OF ANTIBIOTIC RESISTANT STRAINS OF *ESCHERICHIA COLI*

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ABSTRACT

Objective: The objective of the study is to find the bioactivity of few unexplored orchids against antibiotic resistance Escherichia coli (E. coli). Three orchid species namely Aerides odorata, Acampe papilosa & Acampe ochracea that are ethnic to North-East India were investigated for their antimicrobial efficacy against Kanamycin and Ampicillin resistant E. coli.**Methods:**Plants were collected, identified following literature and college herbarium of Karimganj College, Karimganj and shade dried. Extracts were prepared in different solvents in increasing polarity and paper discs soaked in extracts were prepared. Disc plate method was employed to determine the antimicrobial activity of the orchid extracts. The zones of inhibition were measured and interpreted compared to that of available antibiotics.**Results:**Water extract of A. ordata showed 0.5 cm, 0.7 cm & 0.5 cm, A. papilosa showed 0.5 cm, 0.6 cm & 0.5 cm and A. ochracea showed 0.5 cm, 0.8 cm & 0.6 cm inhibition zone against antibiotic susceptible E. coli, ampicillin resistant E. coli & kanamycin resistant E. coli respectively.**Conclusion:**The results suggest that all the three orchid species has good antimicrobial activity against the three strains of E. coli. Although, the work is a preliminary study on antimicrobial screening but the possible efficacy of the three ethnic orchid species opened a new area for antimicrobial drug design research.

Keywords: *Aerides odorata; Acampe papilosa; Acampe ochracea; Escherichia coli; antimicrobial activity; orchids.*

INTRODUCTION

Plants are well known for their medicinal efficacy. Various medicinal aspects of plants have started from the age of Vedas. The ancient medication technique, which comes under Ayurveda, governs the use of plant extract for curing various diseases [1, 2]. In modern technique, the phytochemicals are used for treating various diseases [3, 4].

The chemical bases of plant's medicinal efficacy are due to the presence of the byproduct and end-product of various metabolic processes. These are the secondary metabolites or phytochemicals, which are secreted by plant to defend them from exposed threats of pathogen and predators [3].

North-East India is rich in biodiversity and contains a wide range of orchids and epiphytes. The Barak Valley in Assam has been ornamented by a wide variety of orchids. However, orchids are much-endangered species, but due the endemic floral density of orchid in Assam, these were chosen as the source of plant in the present work.

Antibiotic resistance is a growing problem. Some of this is due to overuse of antibiotics in humans, but some of it is probably due to the use of antibiotics as growth promoters in food of animals. A study published in the journal Science in August 2007 found that the rate of adaptive mutations in *E. coli* is "on the order of 10^{-5} per genome per generation, which is 1,000 times as high as previous estimates," a finding which may have significance for the study and management of bacterial antibiotic resistance [5]. The antibiotic sensitivities of different strains of *E. coli* vary widely. As Gram-negative organisms, *E. coli* are resistant to many antibiotics that are effective against Gram-positive organisms. In the present work, we screen the medicinal efficacy of few orchid species against various resistant strains of *E. coli*.

Hypothesis of the work is presence of number of alkaloids in orchid tissue [6, 7]. Some of them should have efficacious uses. However, the efficacy for producing antimicrobial activity may vary according to species. Similarly, the active principle also may vary from species to species. Screening and selection of most effective one based on experimental results may be useful therapeutically. Conservation and scientific vis-à-vis empirical information based utilization of orchids would repay careful investigation. The major question here is which species of orchid can effectively inhibit the specific pathogens (e.g. *Escherichia coli*). The exact species of orchid for the elimination of pathogens may be selected based on antibacterial activity of the species. The work also focuses on the antimicrobial property of the selected orchids to build the medicinal profile of the orchids, which may be used against other diseases in future. The objective of the work is to develop research to find out drug molecules for combating the threat of emergence of multi-drug resistant pathotypes.

MATERIAL AND METHODS

Collection of materials

Leaves of three orchids were collected from the Karimganj District of Assam without disturbing their natural habitat. The photographs were taken in flowering stage in their natural habitat for identification. The specimens were then identified consulting the herbarium of Karimganj College and standard literature. The orchids collected were identified as *Acampe papilosa* (Figure 1A), *Aerides odorata* (Figure 1B) *& Acampe ochracea* (Figure 1C).

Figure 1: Three selected orchids *Acampe papilosa* (A), *Aerides odorata* (B) and *Acampe ochracea* (C) from Karimganj District, Assam of North Eastern India

Three strains of *E. coli* were selected as test organisms. These were collected from Microbial Type Culture Collection and Gene Bank (MTCC), Indian Institute of Technology, Guwahati (IIT) and Genei (Bangaluru). Among those, the latter two were resistant against Kanamycin & Ampicillin respectively.

Preparation of extracts

Leaves of all the three aforementioned orchids were shade dried, weighed and crushed in water and acetone in the (leaves to solvent) ratio of 2:1 (W/V). The extracts were then filtered and were investigated for their antimicrobial activity. Nutrient agar media with ampicillin and kanamycin suspended separately & nutrient agar for plating and LB broth (Hi-Media) for sub-culturing were used.

Culture and subculture

All the three *E. coli* strains were sub-cultured from their pure culture in LB broth in slanted 10 ml test tubes and incubated at 34±2^{0}C.

Plate culture

Three sets of petridishes (9 cm diameter) were sterilized and were flooded with the LB agar suspended with ampicillin, kanamycin, and nutrient agar separately. The plates were kept in laminar hood until the agar became semisolid. The test organisms were seeded in the respective medium using different inoculation methods.

Antibacterial assay with paper disc

For this assay, the modified paper disc method [8] was followed which uses Whatman No. 1 filter paper for preparation of paper discs soaked in the plant extracts. The soaked discs were allowed to dry overnight. The dried discs were then sterilized under UV light for 15-20 minutes. Sterilized petridishes containing 15 ml LB and nutrient agar were seeded separately with the test organisms in aseptic condition under laminar hood. The sterilized discs were then placed in the petridishes in an ordered manner in aseptic condition. The plates were then incubated at 34±2^{0}C overnight and were examined. The zone diameter were measured and recorded.

RESULTS

The plates were observed and photographs were taken. The plates of control and two resistant variety of *E. coli* in Nutrient agar were shown in Figure 2, Figure 3. The plates with Ampicillin and Kanamycin resistant suspended in Ampicillin and Kanamycin suspended agar were shown in Figure3.

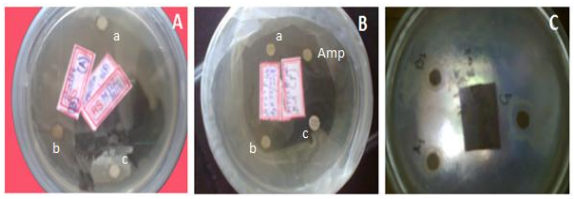

Figure 2: The zone of inhibition of *Acampe papilosa* (a), *Aerides odorata* (b) and *Acampe ochracea* (c) extracts against control (A), ampicillin resistant (B) and kanamycin resistant (C) strains of *E. coli* in nutrient agar

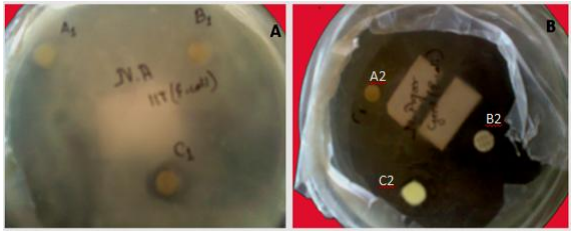

Figure3: The zone of inhibition of *Acampe papilosa* (A1, A2), *Aerides odorata* (B1, B2) and *Acampe ochracea* (C1, C2) extracts against ampicillin resistant (A) and kanamycin resistant (B) strains of *E. coli* in ampicillin suspended and kanamycin suspended nutrient agar respectively.

The zone diameters (in mm) of three set of plates were taken and the mean was tabulated in table 1, 2 and 3 for control in nutrient agar, ampicillin resistant strain in ampicillin suspended agar and kanamycin resistant strain in kanamycin suspended agar respectively and are statistically found significant at $p<0.05$ (Figure 4, Figure 5 and Figure 6).

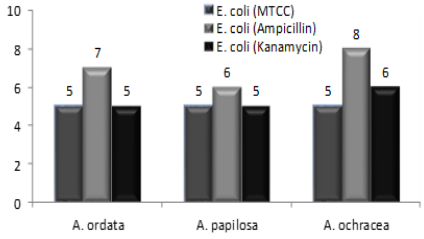

Figure 4: Inhibition zone (mm) showed by water extract of the three orchids *Aerides odorata, Acampe papilosa* and *Acampe ochracea* against nonresistant (MTCC), Ampicillin resistant (Ampicillin) and Kanamycin resistant (Kanamycin) strains of *E. coli* in nutrient agar

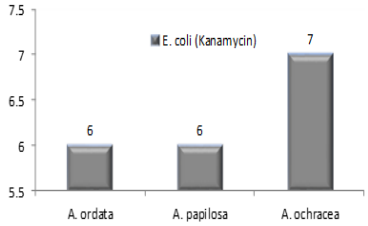

Figure 5: Inhibition zone (mm) showed by acetone extract of the three orchids *Aerides odorata, Acampe papilosa* and *Acampe ochracea* against ampicillin resistant (Ampicillin) strain of *E. coli* in ampicillin suspended LB agar.

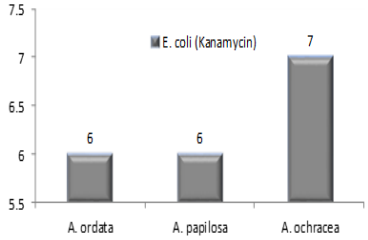

Figure 6: Inhibition zone (mm) showed by water extract of the three orchids *Aerides odorata, Acampe papilosa* and *Acampe ochracea* against kanamycin resistant (Kanamycin) strain of *E. coli* in kanamycin suspended LB agar

The column statistics were used to measure the possible cumulative efficacy of the extracts (Figure 7, Figure 8 and Figure 9).

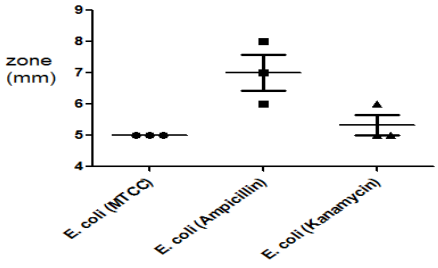

Figure 7: Column statistics analysis of inhibition zone by water extract of the three orchids *Aerides odorata, Acampe papilosa* and *Acampe ochracea* against nonresistant (MTCC), Ampicillin resistant (Ampicillin) and Kanamycin resistant (Kanamycin) strains of *E. coli* in nutrient agar

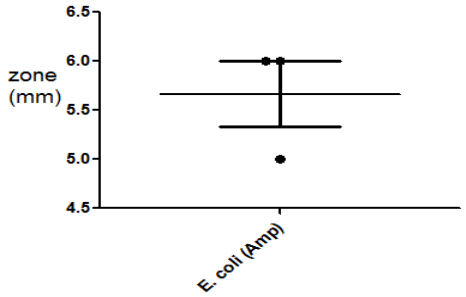

Figure 8: Column statistics analyses of the inhibition zone by acetone extract of the three orchids *Aerides odorata, Acampe papilosa* and *Acampe ochracea* against ampicillin resistant (Amp) strain of *E. coli* in ampicillin suspended LB agar.

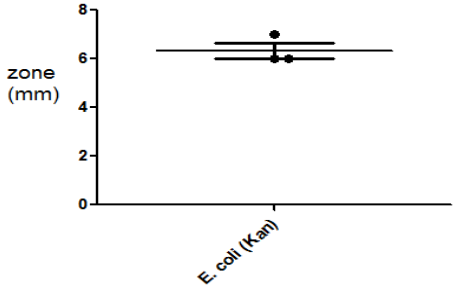

Figure 9: Column statistics analyses of the inhibition zone by water extract of the three orchids *Aerides odorata, Acampe papilosa* and *Acampe ochracea* against Kanamycin resistant (Kan) strain of *E. coli* in Kanamycin suspended LB agar.

Table 1: Antibacterial activity of the *Aerides odorata, Acampe papilosa* and *Acampe ochracea* water extract against nonresistant (E_{nr}), Ampicillin resistant (E_{Amp}) and Kanamycin resistant (E_{Kan}) strains of *E. coli* in nutrient agar using modified paper disc method [8]

Sl	Name of Plants	Leaf extract in	Inhibition zone in mm		
			E_{nr}	E_{Amp}	E_{Kan}
A	*A. ordata*		5	7	5
B	*A. papilosa*	Water	5	6	5
C	*A. ochracea*		5	8	6

Table 2: Antibacterial activity of the *Aerides odorata, Acampe papilosa* and *Acampe ochracea* acetone extract against ampicillin resistant strain (E_{Amp}) of *E. coli* in ampicillin suspended LB agar using modified paper disc method [8]

Sl	Name of Plants	Leaf extract in	Inhibition zone in mm against E_{amp}
A	*A. ordata*		6
B	*A. papilosa*	Acetone	5
C	*A. ochracea*		5

Table 3: Antibacterial activity of the *Aerides odorata, Acampe papilosa* and *Acampe ochracea* water extract against kanamycin resistant strain of *E. coli* (E_{kan}) in kanamycin suspended LB agar using modified paper disc method [8]

Sl	Name of Plants	Leaf extract in	Inhibition zone in mm against E_{kan}
A	*A. ordata*		6
B	*A. papilosa*	Water	6
C	*A. ochracea*		7

DISCUSSION

Plants are always used for establishing new drugs. Among all, orchids have been used as a source of medicine for millennia to treat different diseases and ailments including tuberculosis, paralysis, stomach disorders, chest pain, arthritis, syphilis, jaundice, cholera, leucoderma, diahorrhea, muscular pain, blood dysentery, hepatitis, dyspepsia, bone fractures, rheumatism, asthma, malaria, earache, sexually transmitted diseases, wounds etc. Besides, many orchidaceous preparations are used as contraceptive, cooling agent and remedies in scorpion sting and snakebite [9]. Some of the preparations are supposed to have miraculous curative properties but rare scientific demonstration available, which is a primary requirement for clinical implementations. Due to incredible diversity, high alkaloids and glycosides content, research on orchids is full of potential.

Meanwhile, some novel compounds and drugs, both in phytochemical and pharmacological point of view have been reported from orchids [10, 11, 12]. Linking of the indigenous knowledge to the modern research activities will help to discover new drugs much more effective than contemporary synthetic medicines. In the present investigation leaf extracts of *Aerides odorata, Acampe papilosa & Acampe ochracea* showed antibacterial activity in different solvents. It has been reported that many natural products including pigments, enzymes and bioactive components are soluble in water, which explains the highest yield of extract, while some of the solvents especially acetone are selective for tannins [9]. Different solvents have various degrees of solubility for different phytoconstituents [13].

E. coli is a harmless micro-flora of intestine of higher animals. However, the harmful variety can cause food poisoning leading to diarrhoea and sometime death to the host. Moreover, the *E. coli* can be collected and grown easily and have quite simple genetic makeup, hence these are easy to study and used as a model organism in this work.

The paper disc method is a well-established method [8] among all the antibacterial assay methods and hence employed in this work. Table 1, 2 & 3 showed a remarkable activity (8.0 mm zone diameter of inhibition) showed by the water extract of *A. ochracea*. The activity of the water extracts of *A. ochracea* against *E. coli* (Kanamycin resistant strain grown on LB agar) was 7.0 mm (zone diameter of inhibition). All the three plants showed good antibacterial activity against the three selected strains of *E. coli.* Water extract of showed comparable zone of inhibition against *E. coli* (MTCC), E.coli (ampicillin resistant) & *E. coli* (kanamycin resistant) variety cultured in nutrient agar media respectively. Again, the acetone extract of *A. ordata* showed the highest zone (6.0 mm) of all the three orchids against *E. coli* (ampicillin resistant) variety cultured in ampicillin suspended LB agar media respectively. To the surprise, the water extract of *A. ochracea* showed maximum zone (7.0 mm) against E.coli (kanamycin resistant) variety cultured in kanamycin suspended LB agar media. The growth of respective strains of *E. coli* in ampicillin and kanamycin suspended LB agar showed their resistance property, but the selected orchids inhibited the growth of drug resistant *E. coli* strains showing their high antibacterial efficacy [1, 10, 13-14]. The work also signifies the earlier findings on various antimicrobial activity of orchid [15, 16] and validate the findings that the presence of alkaloid in orchid may be the reason for this efficacy.

CONCLUSION

In the present work, three orchids were selected namely *Aerides ordata, Acampe papilosa* & Acampe *ochracea* for study of the antibacterial efficacy against drug resistant variety i.e., (MTCC), ampicillin resistance & kanamycin resistant variety of *E. coli*, which

is a Gram-negative bacterium. All the three plants showed good antibacterial property against the selected strains of *E. coli*. Among the three strains, the ampicillin resistant variety showed strong inhibition (average zone diameter of 7.0 mm), the kanamycin resistant variety showed comparatively less inhibition (average zone diameter of 5.3 mm) and the (MTCC) variety showed the least (average zone diameter of 5.0 mm). The treatment in the antibiotic suspended medium showed less inhibition for acetone extract (average zone diameter of 5.3 mm) against ampicillin resistant variety than water extract (average zone diameter of 6.3 mm) against kanamycin resistant variety.

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CONFLICT OF INTEREST: None declared.

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