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Ethnomedicinal uses and antibacterial activity of two orchid species collected from Similipal Biosphere Reserve Odisha, India

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Ethnomedicinal uses of orchids among aboriginal tribes of Similipal Biosphere Reserve, India were surveyed. The result indicated that eight species were used as ethnomedicine for treatment of different ailments. Antibacterial activities of crude extracts of two orchid species (*Acampe praemorsa* and *Vanda tessellata*) obtained by four different solvents were studied against some clinically significant human pathogens. The result revealed that all crude extracts showed antibacterial activity in varying degree inhibiting at least one or more test pathogens. Among the solvents, di-ethyl ether extracts showed significant antibacterial activity against all the test pathogens followed by butanolic, chloroform and methanolic extracts. The MIC value of different extracts ranged from 3.5 to 25 mg/ml. The results indicated that the crude extracts were bactericidal in action. The antibiogram pattern of the pathogens revealed multiple antibiotic resistance indexes of 40%-60%. The activity of different extracts was compared with standard antibiotics, in terms of zones of sensitivity. The findings suggest that, ethno-medicinal orchids could be used as an alternative source of therapeutic agent in near future.

Keywords: ethno-medicinal, orchids, tribal community, antibacterial

Introduction

Development of resistance in bacteria against presently available antibiotics is increasingly becoming a global public health concern. Some currently used antibiotics are now unable to eliminate many bacterial infections due to the development of super resistant strains. For this reason, search for new and effective antimicrobial agents has become essential. Natural products from microorganisms are the primary sources of antibiotics. However, with the increasing acceptance of herbal medicine are as an alternative form of health

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care. The screening of medicinal plants for active compounds has become imperative because these may serve as promising sources of novel antibiotic prototypes (Koduru *et al*., 2006). Many common prescribed drugs used now a day are of natural origin (Cragg *et al*., 1997). Hence, the drug discovery process continues to rely considerably on screening extracts or compounds from natural sources. It is expected that, many new drugs from natural products are likely to come from countries which have high biodiversity (Rout, 2004). Unfortunately, despite its commercial interest, the biodiversity-rich nations have limited involvement in such research. Now it has become a necessity that search for new antimicrobial agents from natural sources should involve those plants of ethno-medicinal importance and those countries which have rich biodiversity (Mohapatra and Choudhury, 2005).

Orchids are the largest and most diverse group of plants among the angiosperms (Dressler, 1981). They are mostly cultivated for beautiful flowers which have immense economic importance. However, these are less explored for their medicinal value. But, in many countries like China and in some parts of Europe and America, Australia and Africa, orchids have been used as traditional drugs for a very long time (Dash *et al*., 2008). In India, ethno-medicinal values of several orchids were discussed in "Charaka Samhita", a classic Indian medicinal treatise written by Charaka in Sanskrit, thousand years ago (Kalaiarasan and John, 2012). *Vanda tessellata*, a common orchid has a long history of use by the tribal people of India for its anti-inflammatory properties (Kumar *et al*., 2005, Kumar, 2007). It has been reported that orchids are used in reducing fever, increasing the white blood cell count, curing eye diseases, treating fatigue and headaches and most importantly functioning as anti-cancer agent (Bulpitt, 2005). However, there is meager information on antimicrobial activity of orchids.

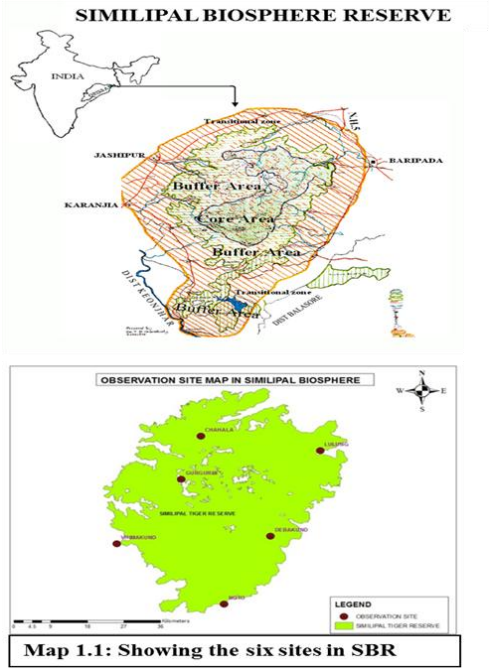
The investigation was therefore directed to document medicinal uses of orchids by indigenous tribal communities of Similipal Biosphere Reserve, India and to evaluation of two commonly used orchid species for their antimicrobial properties.

Materials and methods

Study area

The study was conducted in Similipal Biosphere Reserve (SBR), India located at $21^{o}\ 16^{'}$ to $22^{o}\ 08^{'}$ N latitude and $86^{o}\ 04^{'}$ to $86^{o}\ 37^{'}$ E longitude (Map 1.1). SBR is a rich diversity of flora and fauna, especially medicinal plants, and orchids having elements from Western Ghats and Northeast India. It has fascinating landscape with numerous tribes co-existing in nature including

primitive tribes like "*Santal*", "*Khadias*" and "*Mankadias*". These communities depend on the available natural resources for their livelihood. Ethno-medicinal information and samples were collected from six sites (four in the peripheral and two in the core area) namely Sitakund, Debakunda, Nato, Vimkunda, Gudugudia and Chahala.



Map 1.1: Showing the six sites in SBR

Collection of Information

The data on ethno-medicinal uses of orchids were collected from 2007-2009 by intensive exploration trips conducted over a period of two year. The information on medicinal uses of orchids, their mode of administrations and dosages were gathered by interacting with local people who are actively engaged in health practices and from local Vaidyas or Kabirajs (Dash *et al*., 2008). Additional information such as the vernacular name of the plant, the parts and portions that are used, the mode of preparation and approximate doses and mode of administration were collected. The medicinal uses of the plants were verified from other local Vaidyas by a cross checking method to confirm the authenticity of the information. Photography was made for documentation. Some specimens were deposited in the herbarium of North Orissa University. Ground orchids were potted in the Department garden as living specimens. The specimens were identified through available references (Saxena and Bhramam,

1994; Misra, 2004). Further it was confirmed by a cross checking of herbaria in Botanical Survey of India (BSI, Kolkata).

Selection of plants and Preparation of Extract

Based on ethno-medicinal uses, two commonly used orchid species i.e. *Acampe praemorsa* and *Vanda tessellata* were selected for antimicrobial evaluation. The plant samples were collected from their natural habitat of Similipal Biosphere Reserve, India. The materials (leaves and roots) were shade dried at ambient temperature (31°C) and were powdered using an electric blender. Approximately 10gm of sample, from each were weighed separately and transferred into amber colour bottle containing 100 ml each of organic solvents viz. methanol, chloroform, butanol and diethyl ether. The bottle were sealed and kept on a shaker (Make: Wadegati, Model: 169) for 48 hour and then filtered using Whatman No 1 filter paper. After filtrations the liquid part contain the plant phytochemicals were evaporated to remove the solvents at their respective evaporation temperatures. The weight of the dried extracts was recorded. Each extract was then dissolved in 2 ml of dimethyl sulphoxide (DMSO) for antimicrobial evaluation. The procedure for extraction of crude extracts is shown in Fig. 1.

Test microorganisms

The bacterial strains used in this study were *Bacillus subtilis*, *Escherichia coli* 0157: H7, Entero-pathogenic *Escherichia coli* (EPEC), Entero-toxicogenic *Escherichia coli (ETEC), Shigella boydii*, *Shigella dysentriae*, *Shigella sonnei*, *Staphyloccus aureus*, *Vibrio cholerae* (0139), *Vibrio chlorerae* (Ogawa) and *Vibrio cholerae* (Inaba). The strains were procured from National Institute of Cholera and Enteric Diseases, Kolkata, India.

Determination of antimicrobial activity

The antimicrobial activity of the crude extracts was evaluated by disc diffusion method described by Bauer *et al*. (1966). Overnight cultures (nutrient broth culture) of test bacterial strains were spread uniformly on nutrient agar plates with the help of sterile cotton swabs. Each 6mm diameter Whatman sterile filter paper disc was soaked with 50 µl of any one of the four types of extracts (Section 2.3) through absorption. These extract soaked discs were then placed on the surface of nutrient agar on which bacterial strain was grown. The plates were then incubated at $37°\pm1^0$ C for 24 hour. The results were recorded by measuring the zones of growth inhibition surrounding the disc. Clear

inhibition zones around the discs indicated the presence of antimicrobial activity. All data on antimicrobial activity are the average of triplicate analyses. Minimum inhibitory concentration (MIC) was determined by micro plate dilution method of Eloff (1998) with slight modification.

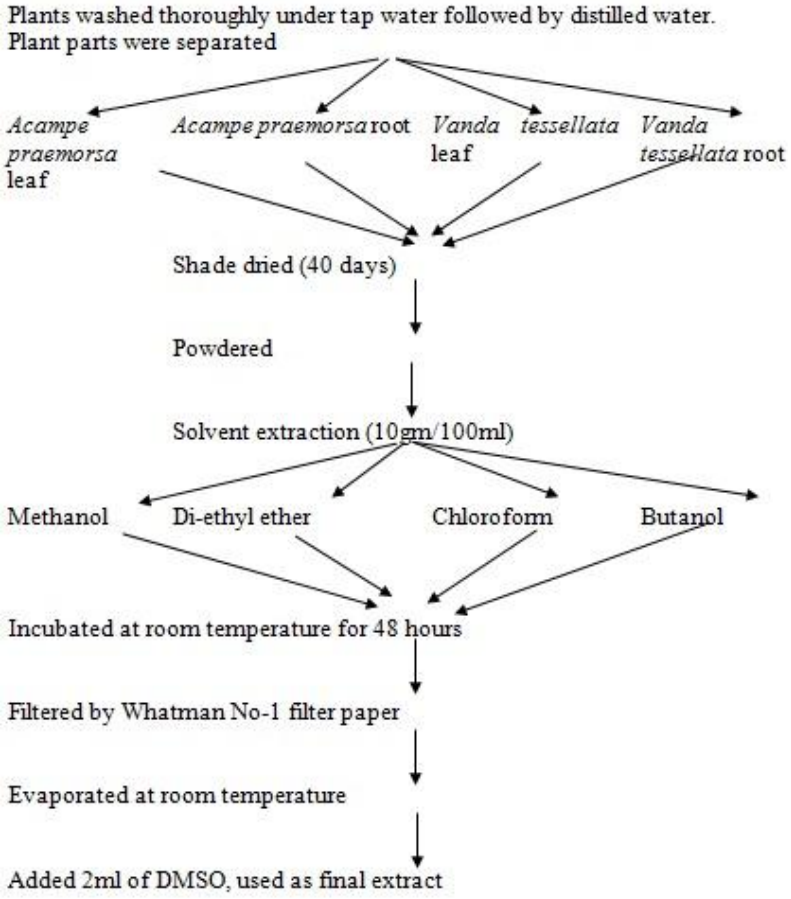


Fig. 1. Flow chart for the extraction of crude extracts from the two Orchid species

Determination of antibiotic sensitivity of the pathogens

Antibiotic sensitivity of the test bacterial strains and their multiple antibiotic resistance (MAR) were determined by following the method of Bauer *et al*. (1966). The antibiotic discs used in this study were Ampicillin (A-10mcg), Nalicidixic acid (Na-30mcg), Tetracycline (T-30mcg), Polymyxin-B (Pb-300u), Chloramphenicol (C-30mcg), Trimethoprin (Tr-10mcg), Ciprofloxacin (Cf-30mcg), Norfloxacin (Nx-10mcg), Sulphadiazine (Sz-300mcg) and Bacitracin(B-30u). All antibiotic discs were procured from Hi-

Media, Mumbai, India. The MAR% of the pathogens was determined by using the following formula:

$$\text{MAR\%}=\frac{\text{Number of antibiotics to which the pathogens showed resistance}}{\text{Total number of antibiotics used}} \times 100$$

Results

Ethno-medicinal uses of orchids

In the present investigation 44 orchid species were documented from the six study sites (data not shown), out of which only 8 orchid species were recorded to be used as ethnomedicines by the indigenous communities. These communities have strong believed on the nature and worship mother earth as "Maa". The orchid species were mostly used for curing different diseases like malaria, stomach pain, diarrhea, insect bites, rheumatism and vitality. The entire information gather from that of number of questionnaires were documented and orchid species with their ethnomedicinal uses were listed in Table 1.

Table 1. Ethno-medicinal uses of some orchids of SBR

Name	Local name	Place of collection	Parts used	*Mode of administration
Acampe praemorsa (Roxb.) Blatt. Et Mc Cann	Rasna	Rani bagan, Lulung, Sitakund	Roots & leaves	The crushed plant material is bandaged on affected regions to cure Rheumatism. Ten drops of warm butter prepared from cow milk is taken on the leaf of plant and bandaged to the legs of the kids to cure tetanus.
Cymbidium aloifolium (L.) Sw.	Pargacha	Debkund, Bangiriposi, Lulung	Leaves & roots	Leaves are heated slightly and the juice is dropped into the ear to cure ear-pain and also the juice is applied on the sites of scorpion bite for relief from pain.
Habenaria sp.	Jibanti	Gudgudia, Sitakund	Tubers	Dried tuber powder with milk enhances the sex power and body stamina.
Geodorum recurvum (Roxb.) Alst.	Siria	Debkund, Gudugudia, Pithabata	Tubers	A mixture of tuber paste with black peeper and 5ml honey mixed is given orally to male for increase the sex power and stamina.
Luisia trichorhiza (W.J. Hook.) Bl.	Khuncha	Pithabata, Sitakund, Bangriposi	Whole plant	Plant is crushed and used for bandage in cramp.
Rhynchostylis retusa (L.) Bl.	Chitra	Baripada, Sitakund, Bangriposi	Roots & leaves	Fresh leaf extracts are applied externally for treatment of wounds and scorpion bite.
Vanda tessellata (Roxb.) W.J. Hook. ex G. Don.	Kani	Bangriposi, Sitakund, Baripada	Leaves	The leaves are crushed and the juice is slightly heated and dropped in the ear for the relief of ear pain.
Vanda testacea (Lindl.) H.G. Rchb.	Madanga	Debkund, Gudugudia, Sitakund	Roots	Root paste is bandaged for treatment of joint pain and rheumatism.

*Data collected, by direct interaction with local People and Vaidyas, through a questionnaire.

Determination of antibacterial activity

Based on ethno-medicinal knowledge and abundance, two orchid species i.e. *Acampe praemorsa* and *Vanda tessellata* were selected for antibacterial study. Crude leaf and root extracts obtained from four different solvents namely, Methanol, Di-ethyl ether, Chloroform and Butanol were used for antibacterial evaluation against 13 pathogenic bacteria. The butanolic extract of *A. praemorsa* leaf showed maximum zone of inhibition against *S. aureus* (15.33±0.53) followed by the *V. tessellata* leaf (15.0±1.0). However, no zone of inhibition was observed against *S. boyedi* (Table 2). Similarly, the chloroform leaf and root extracts of *A. praemorsa* showed highest zone of inhibition against *B. subtilis* and Enteropathogenic *E. coli* respectively but the extracts did not showed activity against Enterotoxigenic *E. coli*, *Vibrio cholera* (inoba) and *Shigella sonnei* (Table 3). Among the solvent Di-ethyl ether extract of both *A. praemorsa* and *V. tessellata* (leaf and root) showed activity against all the test pathogens (Table 4). The methanolic extract showed activity against all the pathogen except *S. sonnei* (Table 5). Among the pathogens *Bacillus subtilis* showed 100% susceptible to all the plant extracts while *Shigella sonnei* was most resistant to the extracts.

Table 2. Antibacterial activity of butanol extract of two orchid species against some human pathogens

Organism	**Butanol extract**			
	APL	APR	VTL	VTR
Shigella dysenteriae	7.33 ±1.53	8.67±1.15	7.33 ±1.53	--
Staphyllococus aureus	15.33±0.58	8.33±1.53	15.0±1.0	--
Bacillus subtilis	13.33±0.58	13.67±1.53	11.67±1.53	12.67±1.53
Enteropathogenic *E.coli*	10.33±1.53	12.33±1.15	9.33±0.58	11±1.0
Enterotoxigenic *E.coli*	10.67±0.58	9.67±1.53	9.67±0.58	9.67±0.58
E. coli O 157:H7	11.33±0.58	14.33±1.53	9.33±0.58	12.33±0.58
Vibrio cholerae (inoba)	13.0±1.0	10.67±2.08	14.67±1.53	11.33±1.53
Vibrio cholerae (ogawa)	11.33±0.58	13.33±1.15	13.0±1.0	10.67±0.58
Salmonella typhi	11.0±1.0	11.33±0.58	--	--
Salmonella typhimurium	13.33±0.58	--	9.67±1.53	--
Shigella boyedi	--	--	--	--
Shigella sonnei	12.0±1.0	11.67±1.53	14.0±1.0	13.67±1.15
Shegella flexeneri	13.0±1.0	13.67±1.53	14.0±1.0	13.0±2.65

-- indicate no activity; ± SD, n = 3

APL: *Acampe praemorsa* leaf; APR: *Acampe praemorsa* root; VTL: *Vanda tessellata* leaf; VTR: *Vanda tessellata* root

Table 3. Antibacterial activity of chloroform extract of two orchid species against some human pathogens

Organism	Chloroform extract			
	APL	APR	VTL	VTR
Shigella dysenteriae	10.67±0.58	12.33±2.08	12.0±1.0	8.33±0.58
Staphyllococus aureus	9.33±1.53	10.67±0.58	9.67±1.53	9.67±0.58
Bacillus subtilis	13.0±1.0	10.33±1.53	10.33±1.53	9.67±1.53
Enteropathogenic *E.coli*	11.0±1.0	13.0±1.0	12.33±1.53	14.0±1.0
Enterotoxigenic *E.coli*	--	--	--	--
E. coli O 157:H7	12.0±1.0	12.33±0.58	11±1.0	12.33±0.58
Vibrio cholerae (inoba)	--	--	--	--
Vibrio cholerae (ogawa)	--	--	10.33±1.53	--
Salmonella typhi	110±1.0	10.33±1.15	11.67	10.33±0.58
Salmonella typhimurium	9.67±1.53	9.0±1.73	10.67±1.53	9.33±1.15
Shigella boyedi	10.67±2.08	9.0±1.0	8.33±0.58	9.0±1.0
Shigella sonnei	--	--	--	--
Shegella flexeneri	12.33±0.58	10.0±1.0	12.0±1.0	11.0±1.0

-- indicate no activity; ± SD, n = 3

APL: *Acampe praemorsa* leaf; APR: *Acampe praemorsa* root; VTL: *Vanda tessellata* leaf; VTR: *Vanda tessellata* root

Table 4. Antibacterial activity of di-ethyl ether extract of two orchid species against some human pathogens

Organism	Di-ethyl ether extract			
	APL	APR	VTL	VTR
Shigella dysenteriae	11±1.0	14.0±1.0	10.0±1.0	11±1.0
Staphyllococus aureus	10.67±1.53	10.0±0.0	12.67±1.53	13.33±0.58
Bacillus subtilis	10.0±1.0	9.67±1.53	9.33±0.58	11.67±1.53
Enteropathogenic *E.coli*	10.67±0.58	12.0±0.0	9.67±1.53	12.33±0.58
Enterotoxigenic *E.coli*	11.0±1.0	11.33±0.58	11.0±1.0	11.33±1.53
E. coli O 157:H7	10.33±0.58	11.0±1.0	11.33±0.58	10.67±0.58
Vibrio cholerae (inoba)	11.0±1.0	13.67±0.58	12.0±1.0	10.33±2.08
Vibrio cholerae (ogawa)	11.0±1.0	11.33±2.08	11.0±1.0	12.33±0.58
Salmonella typhi	10.67±1.15	13.67±1.53	10.0±0.0	11.67±0.58
Salmonella typhimurium	11.67±0.58	10.67±1.53	9.0±1.0	9.33±0.58
Shigella boyedi	12.33±0.58	12.0±1.0	10.33±0.58	12.33±2.08
Shigella sonnei	10.67±0.58	11.67±1.53	11.33±1.53	11.0±0
Shegella flexeneri	12.33±0.58	12.0±1.0	10.67±2.08	12.0±2.0

-- indicate no activity; ± SD, n = 3

APL: *Acampe praemorsa* leaf; APR: *Acampe praemorsa* root; VTL: *Vanda tessellata* leaf; VTR: *Vanda tessellata* root

Table 5. Antibacterial activity of methanolic extract of two orchid species against some human pathogens

Organism	**Methanol extract**			
	APL	APR	VTL	VTR
Shigella dysenteriae	11±1.0	9±1.0	14.33±0.58	11.67±1.53
Staphyllococus aureus	10.67±0.58	12±1.0	13.33±1.53	9.0±1.0
Bacillus subtilis	11±1.0	9.33±0.58	12.33±0.58	10.0±1.0
Enteropathogenic *E.coli*	12±1.0	10.67±0.58	14.33±0.58	11.33±1.53
Enterotoxigenic *E.coli*	11±1.0	9.33±0.58	--	9.67±1.53
E. coli O 157:H7	11±1.0	10.0±1.0	8.67±0.58	9.33±0.58
Vibrio cholerae (inoba)	9.33±0.58	10.0±1.0	--	--
Vibrio cholerae (ogawa)	10.0±1.0	9.67±1.53	--	--
Salmonella typhi	11±1.0	11.33±0.58	10.33±0.58	9.0±0.0
Salmonella typhimurium	9.67±0.58	10.67±0.58	11±1.0	11.33±1.53
Shigella boyedi	9.33±0.58	10.0±1.0	8.0±1.0	9.0±0.0
Shigella sonnei	--	--	--	--
Shegella flexeneri	11±1.0	10.33±1.53	12.0±1.0	9.33±0.58

-- indicate no activity; ± SD, n = 3

APL: *Acampe praemorsa* leaf; APR: *Acampe praemorsa* root; VTL: *Vanda tessellata* leaf; VTR: *Vanda tessellata* root

The percentage of inhibition of both plants extracts (leaf and root) against the test pathogens have been presented in Fig. 2. The methanolic leaf and root extracts of *A. praemorsa* indicated 92.30% of antibacterial activity while that of *V. tessellata* showed 69.23% and 76.92 % antibacterial activity for leaf and root respectively. The chloroform extracts of *V. tessellata* leaf showed 76.92% activity, but leaf and root of *A. praemorsa* and root of *V. tessellata*, showed equal degree (69.29%) of antibacterial activity. Similarly, butanolic extracts of *A. praemorsa* leaf extracts showed 92.30% while the root and *V. tessellata* leaf showed similar degree (84.61%) of inhibition against the pathogens. Butanolic root extract of V. tessellate showed the lowest percentage inhibition (61.53%). Surprisingly, Diethyl-ether extracts of both orchid plants inhibited all the pathogens, representing 100% of antibacterial activity.

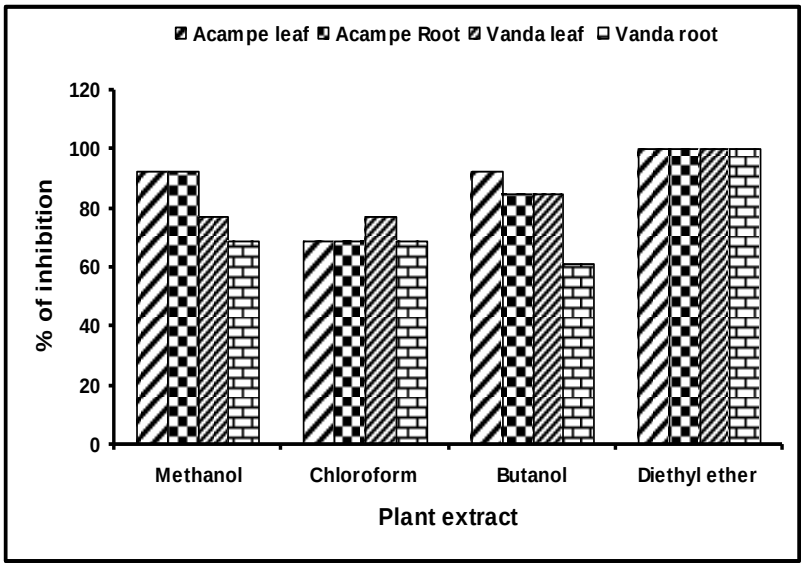


Fig. 2. Depicting percentage of antibacterial activity of *Acampe praemorsa* and *Vanda tessellata* extracts.

MIC value of different extracts

Minimum Inhibitory Concentration values of extracts was performed only for those extracts which showed better antibacterial activity during the preliminary screening by disc diffusion method. The MIC values of different extracts against selected test pathogens are presented in (Table 6). The MIC values of the extract ranged between 3.5-25.0 mg/ml. Both diethyl ether and butanol extracts of *Acampe* root showed lower MIC values against all the pathogens studied that corroborates with our previous studies of Disc Diffusion Method i.e. highest zones of inhibition corresponding to lower MIC values. When the butanol extracts were tested 70% of the test pathogens showed MIC value 7.5mg/ml, and surprisingly butanol extract of *Acampe* root sowed MIC of 3.5 mg/ml against *E. coli* O157: H7. The activity of the extracts reported to be bactericidal as no growth was observed when one loop full of the sample was cultured onto NA plates from the MIC diluted wells, in comparison to control. The chloroform extract of *Acampe* root showed MIC between 6.25 - 12.5 mg/ml against the test pathogens.

Table 6. Minimum Inhibitory Concentration (MIC) of different crude extracts against test pathogens

Organism	*Acampe* leaf	*Acampe* root	*Vanda* leaf	*Vanda* root
Methanol extracts (mg/ml)				
S. dysentriae	10.5	21	10.5	21
S. aureas	10.5	10.5	10.5	21
B. subtilis	10.5	10.5	21	21
E. P. *E. coli*	21	21	10.5	21
S. Flexeneri	10.5	10.5	21	21
*Eco*0157:H7	10.5	21	21	21
Chloroform extracts (mg/ml)				
S. dysentriae	25	6.25	25	25
S. aureas	12.5	12.5	25	25
B. subtilis	25	6.25	12.5	12.5
E. P. *E. coli*	25	12.5	12.5	12.5
S. Flexeneri	25	12.5	25	25
*Eco*0157:H7	12.5	6.25	12.5	12.5
Butanol extracts (mg/ml)				
Vc inoba	7.5	7.5	7.5	15
Vc agawa	15	7.5	7.5	15
S. sonnei	15	7.5	7.5	15
*Eco*0157:H7	7.5	3.75	15	7.5
B. subtilis	7.5	7.5	7.5	7.5
Di-ethyl ether extracts (mg/ml)				
Vc inoba	14	7	14	14
Vc agawa	7	7	14	3.5
S. sonnei	14	7	14	7
*Eco*0157:H7	14	14	7	7
B. subtilis	3.5	3.5	14	14

Antibiogram of the test pathogens

The antibiogram pattern of the pathogens is represented in (Table 7). All the pathogens were susceptible to Chloramphenicol, Ciprofloxacin, Tetracyclin, Polymyxin-B, Norfloxacin and resistant to Ampicillin, Nalicidixic acid, Trimethoprin and Sulphadiazine. Further, it is preferment to mention that MAR index of the pathogens ranged from 40-60%. Furthermore, the zones of inhibition observed by disc diffusion method of different extracts in our investigation can be well compared. Though small zones of inhibition are observed in certain cases, it could be attributable to the impurity of the active principles present in the extracts as well as their poor solubility in different solvents.

Table 7. Antibiogram pattern of the test pathogens

Organism	Zones size in mm*		
	Sensitive to	Resistance to	MAR%
Staphyllococus aureus	C(35), Cf (39), B(27), T(28), Pb(11),Nx(12)	A, Na, Tr, Sz	40%
Bacillus subtilis	C(20), Cf (17), T(14), Na (15), Pb(12), Nx(25)	B, A, Tr, Sz	40%
Vibrio cholerae (inoba)	C(15), Cf (15), T(12), Na(14), Pb(10), Nx(8)	B, A, Tr, Sz	40%
Vibrio cholerae (ogawa)	C(11), Cf (25), T(14), Pb(12),Nx(10)	B, A, Na, Tr, Sz	50%
E. coli O 157:H7	C(20), Cf (25), T(16), Pb(10),Nx(25)	B, A, Na, Tr, Sz	50%
Shigella dysenteriae	C(19), Cf (26), T(12), Pb(13),Nx(24)	B, A, Na, Tr, Sz	50%
Shegella flexeneri	C(25), Cf (15), T(13), Pb(11),Nx(26)	B, A, Na, Tr, Sz	50%
Shigella sonnei	C(22), Cf (26), Pb(8), Nx(12)	B, A, Na, T, Tr, Sz	60%
Enteropathogenic *E.coli*	C(15), T(9), Na(14), Pb(9),Nx(24)	C, B, A, Tr, Sz	50%

Values in parenthesis represent zones of sensitivity in mm. (A: Ampicillin; Na: Nalicidixic acid; T: Tetracycline; Pb: Polymyxin-B; C: Chloramphenicol; Tr: Trimethoprin; Cf: Ciprofloxacin; Nx: Norfloxacin; Sz: Sulphadiazine and B: Bacitracin).

Discussion

Traditional knowledge has been used since time immemorial by indigenous and local communities and plays an important role in treatment of various ailments. Many of the *orchids* are used as medicine by the *tribal communities and* currently being used as drugs in the Indian system of medicine. In the present investigation it have been found that tribal communities of Similipal Biosphere Reserve uses eight different species of orchids along with the other medicinal plants for treatment of different diseases. In many instances, it was found that they mostly use the orchid species, *Vanda tessellata* for reliving ear pain (otomycosis), stomach pain and for treatment of rheumatism. There were also several reports on the ethonomedicinal property of orchids in India and some Asian countries like Nepal, China, Thailand, Japan etc., which were very similar to our present investigation (Tiwari *et al*., 2012; Medhi and Chakrabrti, 2009). Similar to our work, Wantanabe *et al*. (2007) reported the use of *Cymbidium goeringii* for treatment of hypertensive and diuretic activates. In China and Japan the leaves of *Dendrobium densiflorum* and *D. fimbriatum* were used for promoting the production of body fluid (Fan *et*

al., 2001; Bi *et al*., 2003). Furthermore, Liu and Zhao (2003) reported that *D. nobile* was used as tonic to nourish stomach, promote production of body fluid and reduce fever in China. Moreover, leaf juices of *Vanda roxburghii* and *V. tessellata* were used for treatment of otitis and certain inflammatory conditions (Chawal *et al*., 1992; Suresh *et al*., 2000).

Out of this eight species, *Acampe praemorsa* and *Vanda tessellata* were selected for studying their antimicrobial potencies, as these two species are commonly distributed in this area. Methanolic, Butanolic, Chloroform and Di-ethyl ether extracts of root and leaf of these two species were screened for their antibacterial activities against 13 human pathogens primarily by Disc Diffusion Method. The result indicated that the crude extracts showed activity inhibiting at least of one of the test pathogens. The overall preliminary screening showed that gram positive bacteria were more susceptible to gram negative bacterial pathogens. Similar result was obtained by Martin, (1995) who attributed that it could be due to presence of their thick murein layer in gram negative bacteria, which prevents the entry of inhibitors. The Gram-positive bacteria on the other hand are more susceptible because the peptidoglycan constituting the outer layer is not an effective permeability barrier. Therefore, the walls of Gram-negative organisms are more complex than the Gram positive ones. The walls act as a diffusion barrier making Gram-negative bacteria less susceptible to the antimicrobial agents than are Gram-positive bacteria (Nostro *et al*., 2000; Hodges, 2002).

Since the Development of drug resistance among the clinical pathogens is of global concern today, and each antibiotic has its own limitations regarding their safety margin. In this investigation we reported a high MAR% index i.e. 40-60% among the common human pathogens studied. Therefore, there is an urgent need for search of newer antibacterial agents from natural sources.

Several reports are available in literature regarding the antimicrobial activity of medicinal and aromatic plants; more or less few botanicals are in chemotherapeutic use. However, to the best of our knowledge, the plants used in this investigation (Orchid species: *Acampe praemorsa & Vanda tessellata*) are not reported in literature regarding their antimicrobial properties. Here we reported antibacterial activity of different extracts against human pathogens through primary screening methods as well as through secondary methods (Determination of MIC of extracts). The activity of the extracts observed to be bactericidal in nature. Further, the activity of these extracts in terms of zones of inhibition is comparable with standard antibiotics, is indicative of their potential for commercial exploration.

Conclusion

Recently there has been tremendous progress in medicinal plants research but orchids have not been exploited fully for their medicinal application. The present finding indicated that orchid species could be potential sources of antimicrobial agent. Although the antimicrobial analysis of both the species is a preliminary work but necessary for their better utilization as therapeutic agents. The future investigations should be designed to isolate the active principles through different techniques to unravel the mystery existing in the system. Studies such as this are a pre-requisite for tapping the antimicrobial potential of these natural products.

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