Antimicrobial activity of *bulbophyllum kaitense*. Rechib. Stem of eastern penisular flora in india.

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Abstract: The present investigation was carried out to evaluate the antimicrobial activity of *Bulbophyllum Kaitense*. The kolli hills a part of Eastern penesulor flora of India is a treasure of medicinal plants. An attempt has been made together for the information about the traditional use of herbs from the local healers. *Bulbophyllum Kaitense* stem is being used for the treatment of certain anticancer. Antioxidant, Antiinflammatory Nematicide, pesticide Lubricant Anti and rogenic and Antimicrobial activity to various diseases as medicine.The plant parts of stem are collected and phytochemicals present in them are analyzed . Hence stem is selected as investigation material. Antimicrobial activity of the various solvent extracts (Petrolieum either, chloroform, ethanol and aqueous) are screened for both samples of the 16 organisms investigation, streptococcus pneumoniae, Bacillus subtilis, Salmonella typhi, Salmonella paratyphi, Pseudomonas aeruginosa, Escherichia coli, Kolebsilla pneumoniae, Entrobacter facalis, Shigella flexneri, Micro coccus specieas and fungal organisms. Aspergillus fumicatus, Aspergillus flavus, Aspergillus niger, Microsporum gypseum. Tricho phyton rubrum and mucor specieas are found to be sensitive to life extracts. The fungi organisms are found to be more sensitive than Gram – Positive bacteria and Gram-Negative bacteria. The inhibition is found to be more in ethanol and chloroform extract. This research proves that *Bulphyllum kaitense*. would be the herbal medicine and stem can be used as herbal and scientific medicine throughout in treating microbial infections in humans.It is indification of bioactive compounds present in the *Bulphyllum kaitense* stem. It is the first report of investigation for Antimicrobial activity in world.

[A.Kalaiarasan, P.Kumar, S.Ahmed john**. Antimicrobial Activity of *Bulbophyllum Kaitense*. Rechib. Stem Eastern Penisular Flora in India.** Journal of Nature and Science 2012;10(2):41-44]. (ISSN: 1545-0740**).**
http://www.sciencepub.net. 7

Keywords: *Bulphyllum kaitense*, Antimicrobial activity, Phytochemicals, Extract.

1. Introduction

Traditional medicine has a long history of serving to the peoples all over the world. Phytochemicals of medicinal plants serve a lead compounds is drug discovery and design. Medicinal plants are rich source of novel drugs that forms the ingredients is traditional systems of medicine, modern medicines, pharmaceutical intermediates, bioactive principles and lead compounds in synthetic drugs (Ncube, 2008).

The enthnobotany and ubiquitous plant provide a rich resource for natural drug research and development. In recent years, the use of traditional, medicine information on plant research has again received considerable interest. Mean while, the need for basic scientific investigations on medical plants using indigenous medical systems becomes imminent. He has also been very well documentd in the world forum by WHO's report stating that more than 80% of world's population are dependent on plants to meet their primary health care needs (Ahmadullah and Nayar, 1999) plant metabolites have been of great interest to man for a long time due to their pharmacological relvance (Arora, Kaur and Kaur, 2003). A large proportion of world population, especially in the developing countries depends on the traditional system of medicine for a variety of diseases. Plant products are also a source of very potent and powerful drugs that have stood the test of times and modern chemistry has not been able to replace most of them. Plant based drugs constitute a major share of all the officially recognized systems of health in India, China viz., Ayurveda, yoga, unani, siddha, homeopathy and naturopathy, except allopathy (Vaidya and Devasagayam, 2007). To understand the mechanism of action, the researchs have worked at molecular levels and several significant phytochemicals have been isolated (Singh and Malhotra, 2005). In many countries including India and china, thousands of tribal communities still use folk here medicinal plants for the cure of various diseases. It has been confirmed by WHO, that traditional medicines, based largely on different species of plants and animals serve the health needs of large number of people; especially for millions of people in the vast rural areas of developing countries. (Kong et al., 2003).

The orchids are one of the largest groups of Angisperms belonging to the family Orchidaceae. They occur in diverse habitat conditions of our country. There are about 20,000 species grouped about 800 genera distributed all over the world except polar region and dry deserts. India is one of the richest orchid habitats with about 2500 species in

167 genera represented in six sub families,17 tribes and 30 sub tribes. Various phytogeographical situation accompanied by the variation in elevation, temperature, rain fall and humidity have contributed to the rich diversity of orchids in India. The orchids have been described as the 'Royal family' and contain their own specialized characters such as intriguing flowers, exciting colours, varied shapes and great diversity of growth habitats would certainly agree that they are rather special plants (Hedge, 1997) in recent years the group has come under renewed focus for two major reasons: many of the beautiful orchids are threatened due to over exploitation, the plants have tremendous scope in horticulture and pharmacognosy, but this potential has remain largely untapped in India (Pathak et al., 2001).

Yet only in the later part of the 20th Century, the medicinal value of Orchid has been recognized. In the Asthavarga is in portant ingredient of various classical Ayurvedic formulations like chavyanprasa four have reported to be orchid Jivaka, (Malaxis muscifrea) Rishbhaka (M.Acuminata) Riddhi (Habenaria intermedia) Vriddhi (H.Edgeworthii) Traditional chinese medicine widely utilized orchids in medicines. In India work has been carried out on chemical analysis of some medicinally usful orchids. Eulophia campestris, ochis latifolia and vanda roxburgii are some important plants.(Amritpal Singh, and Sanjiv Duggal.2009).

Folk medicine has reported *Bulbophyllum kaitense* a terrestrial orchid. This is an epiphytic family orchidaceae Endemic to south India. The plant is not very common is south India. The plant is dense maths on tress and rock. This is native of India occurs in the forest of estern penesulor flora of Kolli hills above 1300 m. Sympodial epiphytes with uninodal pseudobulb. Inflorescense, umbellate scape pseudobulbs greenish. Sub fusiform not angled 2Cm long 4-5 cm part on the Zone leaves 9-13 cm long flowers without mentum. Sepal unequal petals shorter than the lateral sepals. (Mathtthew.1981).

The plants have been used in the indigenous medicine. This information was gathered by questioning local healers and knowledge able villages of the Kolhi hills. A part of the Eastern penesulor flora (South India). The Information that a plant is used by traditional healers indicates that it is fertile area for scientific investigation.

2. Materials and Methods

Collection of plant materials

The healthy plant materials of Bulbophyllum Kaitense. Recheieb. Were collected from the kolli hills. It was authenticated by Ret, Dr.S.John Britto, The Director, The Rabinat Herbarium, St.Joseph's College, Tiruchirappalli, TamilNadu. India with the help of herbarium record. The plant voucher number: RHT.872.The Kolli hills, one among the hills of the Eastern Penesular flora has it geographical position between 11°.10' 00" to 11°.30' 00" N and 78°.15' 00" to 78°.30' 00" belongs to Namakkal district and it is situated in two taluks namely Namakkal and Rasipuram, covering a total area of about 503Sq.Km. The attitude is ranging for 200-1415m about MSL from the foothills. The mean maximum and minimum temperature is 35° C and 18° C respectively (HariKrishnan, 1977). The air dried plant powders (100 g) were successively extracted with Petroleum ether, Chloroform, ethanol and aqeous using a oxhlet apparatus. The extracts so collected were distilled off on a water bath at atmospheric pressure, and the last trace solvents were removed in vacuo.

Preliminary phytochemicals screening

Phytochemical tests were carried out for the solvent extracts. Petroleum ether, chloro form. Ethanol and ageous extracts were subjected to routine qualitative chemical analysis to identify the nature of phytochemical constituents. Standard procedures were followed to identify the described by sofowara (1993), Harborne (1973) and Brindha et al., (1982).

1. **Test for Terpenoids**

5 ml of the extract was mixed with 2 ml of chloroform and concentrated sulphuric acid to form a layer. A reddish brown coloration in the interface showed the presence of terpenoids.

2. **Test for flavonoids**

5 ml of the diluted ammonia solution a portion of the aqueous extract was added, followed by addition of concentrated suphuric acid. Appearance of yellow coloration indicates the presence of flavonoids.

3. **Test for Reducing Sugars**

2 ml of test solution was added with a 2 ml Fehling's reagent. A (or) B. and 2 ml of water formation of reddish orange color indicates the presence of reducing sugar.

4. **Test for Phenols**

2 ml of test solution in alcohol was added with one drop of neutral ferric chloride 5% solution. Formation of intense blue color indicates the presence of phenols.

5. **Test for Catechins**

2 ml of test solution in alcohol was added with Ehrich reagent and a few drops of concentrated HCl formation of pink color indicate the presence of catechins.

6. **Test for sapomins**

2 ml of test solution was added with H_2O and shacked formation of foamy eather indicates the prsence of saponins.

7. Test for Tanins

2 ml of test solution was added with H_2O and head acetate. Formation of while precipitate indicates the presence of tannins.

8. Test for Anthroquinone

2 ml of test solution was added with magnesium acetate. Formation of pink color indicates the presence of Anthroquinones.

9. Test for Quinine

1 ml of extract, 1 ml of concentrated sulphuric acid was added and was allowed to and for some time to develop color. Development of red color shows the presence of quinine.

10. Test for Coumarin

1 ml of extract, 1 ml of 10% NaOH was added and was allowed to stand for some time development of yellow color shows the presence of coumarin.

11. Test for Glycosides

1 ml of the extract, 1 ml of alpha napthol was added to which chloroform was added along the sides and it was looked for the development of color and the result was recorded. Development of Violet color indicates the presence of glycosides.

12. Test for Carbohydrate

Agueous or alcoholic solution of substance was added with 10% aqueous solution of alpha Napthol shaken and added concentrates sulphuric acid along the side of the side of the tuve. Violet ring at the Junction of two liquids shows presence of Carbohydrates.

13. Test for Sugar

0.5 ml of the Filtrate. 0.5 ml Benedict's reagent was added. The mixture was heated on boiling water both for 2 minutes. A characterisets of red coloured precipitate shows presence of sugar.

Antimicrobial Activity

The extracts were tested for the antibacterial activity. The microbial strains employed in the biological assays were Gram-Positive bacterial Streptococcus pneumoniae (MTCC 2672), Bacillus subtilis (MTCC 441), Gram- negative bacteria: Salmonell typhi (MTCC 734) Salmonella paratyphi (MTCC 735) Pseudomonas aeruginosa (MTCC 2474) Escherichia coli (MTCC 119), Klebsilla pneumoniae (MTCC 3040), Entrobacter facalis, Shigella flexneri, Micrococcus specieas, Fungal strains: Asperigillus fumicatus (MTCC 2584), Trichophyton rubrum (MTCC 296). Microsporum gypseum (MTCC 2819) Aspergillus flavors (MTCC 2813), Aspergillus Niger (MTCC 2612) Mucor Specieas.

Determination of antibacterial activity

Agar well diffusion assay

Agar well diffusion method was followed. Muller-Hinton Agar (MHA) plates were swabbed (Sterile cotton Swabs) with 8-12 h old brothe cultures of the respective bacteria. Sterile circular steel was used to make wells, each measuring 8mm diameter, in each of the plates. About 0.3 ml each of 50, 25, 12.5 and 6.25 mg/ml of concentrated test sample swith DMSO was added into the wells using sterilized dropping micropipettes and allowed for diffusion at room temperature for 2h. The plates were incubated at 37°C for 24 h. The solvent without extracts served. After 24 h of incubation, diameter of the inhibition zone was recorded in mm. The experiment was repeated thrice and the average values were calculated for antibacterial activity.

Determination of antifungal activity

Agar well diffusion method was followed but nutrient medium used was Sabouraud Dextrose Agar (SDA). The Sabouraud Dextrose Agar plates were swabbed (Sterile Cotton Swabs) with 8 h old broth culture of the respective fungi. A sterile cork borer was used to place four wells, each measuring 8 mm in diameter, in each of the plates, about 0.1 ml each of 50, 25, 12.5 and 6.25 mg/ml of concentrated test samples with DMSO was added into the wells using sterilized dropping micro pipettes and allowed for diffusion at room temperature for 2h. The plates were incubated at 28°C for 18-24 h. Diameter of the inhibition zones was recorded the experiment was repeated thrice and the average values were calculated for antifungal activity.

Results

Preliminary phytochemical analysis of various solvent extracts such as petroleum ether. Chloroform, ethanol and aqueous of the Bulbophyllum Kaitense. Rechieb stem recorded in the (Table1) Terphenoids, Flavonoids, Saponins, Tanins, Coumarin, and Quinine. Carbohydrates were present. The ethanol extract alone contains Tamins, coumarin, quinine. The petroleum ether extract alone contains Terpenoids, Tanins, Coumarin and Carbohydrates. Different solvent extracts of *Bulbophyllum kaitense* stem were tested antibacterial activity. The values were recorded and averaged (Table: 2). Different solvent extracts of *Bulbophyllum kaitense* such as petroleum ether, chloroform ethanol and aqeous were tested. Fungi organisms were highly sensitive than Gram positive bacteria and Gram negative bacteria in tested plant extracts. The zone of inhibition ranging from (14mm - 34mm) against Microsporum gypseum. Aspergilus nigiri, Mucor sp., Aspergilus fumicatus recorded in ethanol and

chloroform extracts. Were expressed more or less similar activity in petroleum ether, and aqueous extracts.The *Bulbophyllum kaitense* stem were tested in Antibacterial activity. The values were recorded and averaged (Tabel: 2) different solvent extracts.The plant were tested Gram-Negative bacteria were highly sensitive than gram positive bacteria in tested plant extracts. The zone of inhibition (ranging from 11mm-29mm) against micrococcus specieas, shigella flexneri, Entrobacter facalis, Klebsilla pnemonia, Salmonella typhi, Salmonella paratyphi, Stroptococcus pnemoniae. Bacillus subtilis and Stroptococcus pneumoniae, Antimicrobial activity showed more activity than chloroform, ethanol and aqueous. Extracts in the respectively (Table: 2) where as less activity recorded in petroleum ether.

Discussions

The current investigation clearly indicated maximum activity was observed from the *Bulbophyllum kaitense* stem extract was potent against Ten tested bacterial strains depended manner. There are effectiveness of traditional herbs against gram positive and gram negative microorganisms and as a result; plants are still recognized as the bedrock for modern medicine to treat infection diseases. However, it was more active against fungal strains. The potential of compounds against the standard strains may be explored in order to develop therapeutics for micro organism. The results of the current to investigation provide scientific support for the claims of the ethmic uses of the medicinal plant. Discovery of new drugs for various diseases such as skin. Tooth ache diarrhea and other microbial infections are possible if active priniciples from solvent extracts are isolated and tested pharmacologically and clinically.

Conclusion

The greatest service which can be rendered any country is to add a useful plant to its culture. “Plants have forever been a catalyst for our healing. In order to halt the trend of increased emerging and resistant infections disease. It will require a multi pronged approach that includes the development of new drugs. Using plants as the inspiration for new drugs provides an infusion of novel compounds or substances for healing disease. Evaluating plants from the traditional Indian system of medicine provides us with clues as to how these plants can be used in the treatment of disease. Many of the plants presented here show very promising activity in the area of antimicrobial agents.

Acknowledgement

I would like to thank whole heartedly Dr. S. Ahmed John Professor and Head. Department of Botany. Jamal Mohamed College, Tiruchirappalli-620 020. Tamil Nadu, India for guiding and supporting me, throughout course, of this investigation.

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11/12/2011