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Anitmicrobial activity of *Vanilla planifolia*

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Abstract: *Vanilla planifolia* is a popular orchid species and the present study investigates its antimicrobial activity against few pathogenic bacteria. The active ingredient of the *Vanilla* extract was resolved by HPLC and the compounds identified were flavonoid and alkaloid in nature.

Keywords: Orchid-*Vanilla planifolia*, antimicrobial activity, HPLC.

Introduction

Infections caused by pathogenic bacteria and fungi are increasingly recognized as an emerging threat to public health (Walsh *et al*., 1996; Wu, 1999). Although many drugs are available at present for the treatment of infectious diseases, their use is limited by a number of factors such as low potency, poor solubility, emergence of drug resistance strains and drug toxicity (Kaufman, 2000; John, 2002). The antibiotic use is not successful always since many of the pathogenic microbes, especially bacteria and fungi have developed substantial resistance to the antimicrobial drugs (Jones, 1998; Sushilkumar, 1998; Austin *et al*., 1999). This may be due to selective pressure on antibiotic-sensitive organisms from the population with the consequent increase in the number of resistant ones. While such a development poses a serious threat to public and a great challenge to physicians, most affected are the under-developed as well as developed countries (Jones, 1998).

Decades ago in India, typhoid could be cured with three inexpensive drugs namely Cephalosporins, Penicillin-G and Chloramphenicol. But today these drugs are largely ineffective against the life threatening typhoid fever. Therefore there is an urgent need for the discovery of alternative, safer and more effective antimicrobial agents in order to control the life threatening pathogens, which has brought in the attention of biologically active compounds from plant and animal species (Arokiyaraj *et al*., 2008; Gangadevi *et al*, 2008; Rehan Ahmad *et al*., 2008; Chellaram & Edward, 2009). Plants are the oldest source of pharmacologically active compounds, and have provided humankind with many medically useful compounds for centuries (Cordell, 1981). Among the available 250,000 to 500,000 species of plants on earth, only 1 to 10% of these are used as food by both human and animal species. It is possible that even more can be utilized for medicinal purposes (Moerman, 1996). Hippocrates (in the late fifth century BC) has already mentioned 300 to 400 medicinal plants (Schultes, 1978). It is estimated that more than two thirds of the world's population relies on plant derived drugs. Some 7000 medicinal compounds used in the Western pharmacopoeia are derived from plants (Caufield, 1991).

The Bioactive compounds in plants are produced as secondary metabolites. Examples include alkaloids, proteins (Chakraborty & Brantner, 1999), which may be stage specific or organ specific or tissue specific. In fact there are several studies which have revealed the presence of such compounds with antimicrobial properties (Cowan, 1999).

Numerous orchid species are traditionally used in herbal medicine as a remedy for microbial infections and number of other ailments. However, the potential of most of the orchid species for therapeutic use is yet to be scientifically explored. The use of orchids as herbal medicine has a very long history with many of their species being used as medicine in different countries for their therapeutic uses. As early as 200 BC the Chinese *Materia medica* (Shong Nung Pen - Gsao ching) mentions Dendrobium as a source of tonic, astringent, analgesic and anti-inflammatory substance. *Vanilla planifolia* is a commercial as well as medicinal orchid belonging to Orchidaceae family. The extract of this plant is used in treating hysteria, rheumatism and other low forms of fever. The principal constituent of *Vanilla planifolia* is vanillin, a methylprotocatechuic aldehyde which occurs in Vanilla beans. It is also used as a major flavouring agent for medicinal syrups. In addition, this orchid contains alkaloids, flavonoids, glycosides, carbohydrates and other phytochemicals. However, this plant has not been scientifically validated for its antimicrobial activity. Hence, the current study was initiated to determine antimicrobial potential of *Vanilla planifolia* against different pathogenic microbes.

Materials and methods

Preparation of plant extracts

The plants were collected from their natural habitats namely, from Kolli hills, Nammakal district in Tamil Nadu (Plate 1). The plants (leaf & stem) were shade-dried at ambient temperature (31 ºC) and were powdered using an electronic blender. Following which the aqueous extract was prepared by suspending hundred grams of dried powder in 600ml of sterile double distilled water. Subsequently, solvent extracts were prepared by soaking the powdered material in 600 ml of each of the solvents *viz*. ethanol, choloroform, ethylacetate and petroleum ether in a soxhlet apparatus for 72 hr at 31 ºC until complete extraction.

Test Microorganisms

The bacterial strains used for the screening were *Escheriachia coli* and its mutant K12 , *Proteus vulgaris*, *Enterobacter aerogens*, *Bacillus cereus, Streptococcus faecalis*, *Klebsiella Pneumoniae*, *Salmonella typhi*,

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Antibacterial activity of Vanilla planifolia (category 1=leaf, category 2=stem) assessed by disc diffusion method. Series 1. Aqueous, Series 2. Ethanol, Series 3. Chloroform, Series 4. Pet Ether: Ethanol (1:1).

Fig.1 (against *E.coli*)

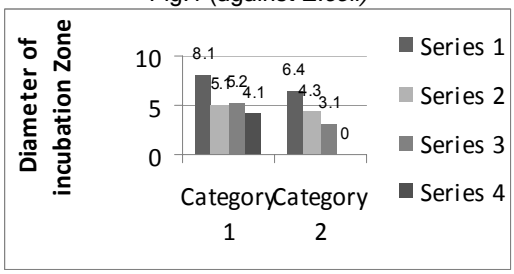


Fig.2 (against K_{12})

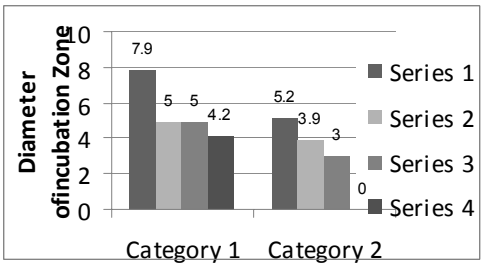


Fig.3 (against *Proteus vulgaris*)

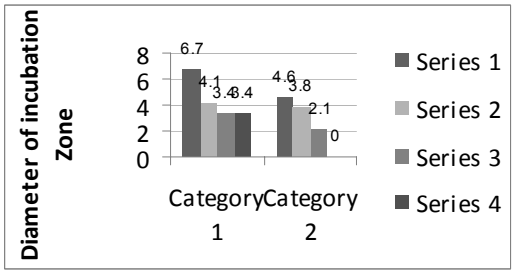


Fig.4 (against *Enterobacter aerogens*)

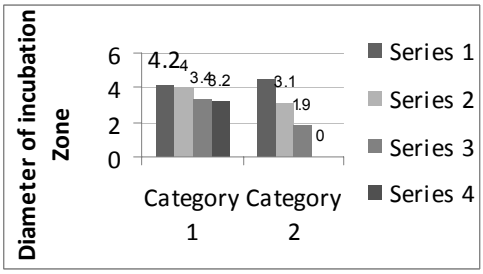


Fig.5 (against *Serratia marcescens*)

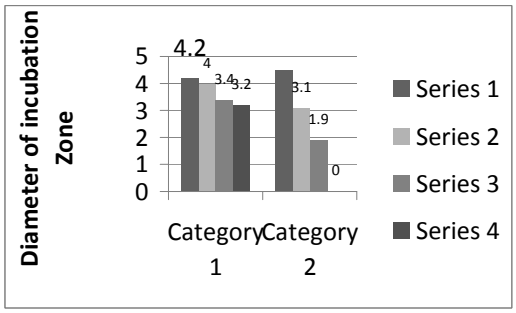


Fig.6 (against *Pseudomonas aeruginosa*)

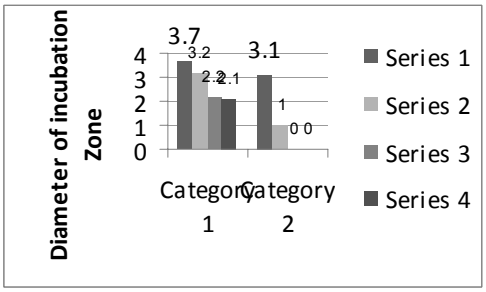


Fig.7 (against *Klebsiella pneumonia*)

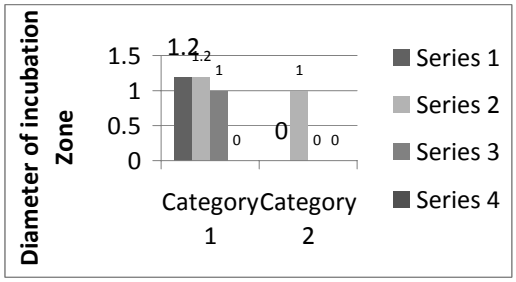


Fig.8 (against *Salmonella typhi*)

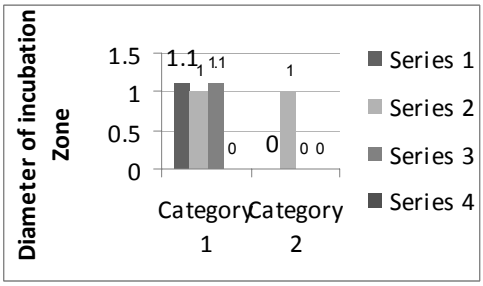


Fig.9 (against *Bacillus cereus*)

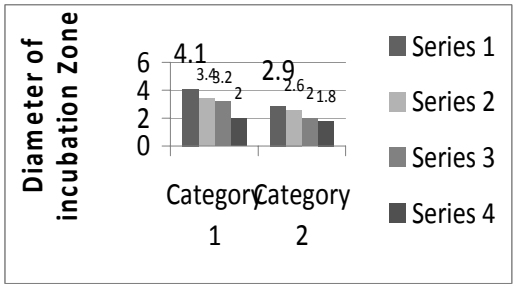
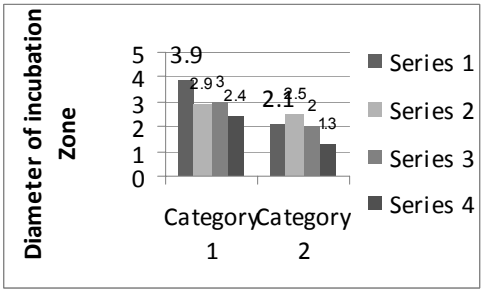


Fig.10 (against *Bacillus cereus*)



Serratia marcescens and *Pseudomonas aeruginosa*. The bacterial strains were procured from Department of Microbiology, Institute of Basic Medical Sciences, University of Madras, Chennai and also from Microbial Type Culture Collection Centre, Chandigarh, Punjab. The obtained bacterial cultures were maintained in Nutrient Agar (Hi-media Laboratories Pvt. Ltd., Mumbai) and on Potato Dextrose Agar slants, respectively (Hi-medicinal Laboratories Pvt. Lt., Mumbai).

Determination of antimicrobial activity

The antimicrobial activity of the aqueous and other solvent extracts of *Vanilla planifolia* (leaf, stem) was evaluated by Disc-diffusion method (Maruzzella & Henry, 1958).

Phytochemical screening for qualitative chemical examination

The extracts obtained were subjected to qualitative tests for identification of chemical constituents of the selected parts of the plant (Brindha *&* Saraswathi, 1981). One hundred gram of the dried, powdered form of each plant part (root, leaf, fruit and seeds) were separately extracted with petroleum ether (40-60 ºC), benzene, chloroform and ethanol in 150 ml of each for six hrs in a soxhlet apparatus. These extract were

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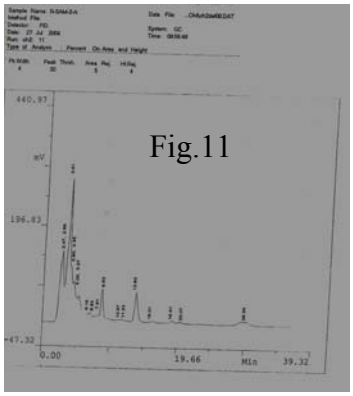

Fig.11

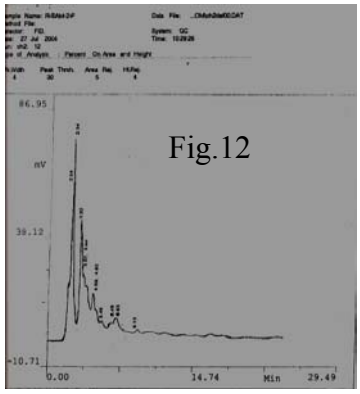

Fig.12

concentrated and further used for preliminary phytochemical screening (Table 1).

HPLC analysis

The chemical composition of the plant extract as resolved by HPLC pertaining to flavanoids and alkaloids are provided in Table 2.

Results and discussion

Disc diffusion assay

The results of antibacterial sensitivity of various solvent extracts of *Vanilla planifolia* (leaf, stem) by disc diffusion method are in depicted Fig. 1-10. The results reveal that both leaf and stem extracts are potent antimicrobials againsts all the pathogenic organisms studied. The antibacterial activity was screened from the zone of inhibition. Among various solvent extracts studied, ethanolic leaf extract showed higher degree of inhibition followed by ethylacetate and chloroform. In the present study, the antibacterial sensitivity was maximum in ethanolic leaf and lowest inhibition was observed in petroleum ether. The aqueous extract did not show any antibacterial activity. The diameter of inhibition zones for each of the samples were compared with standard antibiotic (chloramphenicol 30 mcg/disc). It was noted that the inhibition zones of the samples differed from the inhibition zones of standard antibiotic. The leaf extracts exhibited higher degree of inhibition than the stem extracts. The diameter of the inhibition zones were noted in the ethanolic leaf extracts (Table 3) showed significant inhibition against the pathogens studied. The inhibition zone was found to be higher against *E.coli* (8.1 mm), *E.coli* mutant K_{12} (7.9 mm), and *Proteus vulgaris* (6.7 mm). Moderate antibacterial activity was observed against *Enterobacter aerogens* (4.2 mm), *Streptococcus feacalis* (3.9 mm), *Pseudomonas aeruginosa* (3.7 mm) whereas, low degree of inhibition zones were noted against *Klebsiella pneumonia* and *Salmonella typhi*. It is surprising to record that *Pseudomonas aeruginosa* which is known to be a most resistant bacterium, even to synthetic antibacterial drugs was found to be sensitive to the leaf (ethanol) extract of *Vanilla*. Similarly, *Salmonella typhi*, a multidrug resistant bacterium was also susceptible to the leaf extracts (ethanol). The results of various solvent extracts of stem of *Vanilla planifolia* exhibited inhibitory action against all the bacteria, but the inhibition zones were less than the standard antibiotics. Absence of inhibition zones was also noted in some of the extracts *viz* aqueous and petroleum ether, ethanol extract. The ethanol extract of *Vanilla planifolia* effectively inhibited the growth of both the gram-positive and gram-negative bacteria studied. Similar results were also reported by few workers (Grierson *et al*., 1999) whereby, majority of the antibacterial activity was observed in the ethanolic extracts. Aqeous extracts showed nil activity. These results are in accordance with the earlier reports (Chakrabarty & Branter, 1999; Aburajai *et al.,* 2001).

Table 1. Preliminary phytochemichal tests (Brindha et al., 1981)

Test	Observation	Inference
Test solution+ minimum quantity of choloroform+3-4 drops of acetic anhydride and one drop of concentrated sulphuric acid	Purple color changed to blue or green	Presence of steroids
Test solution + piece of tin + 3 drops of thionyl chloride	Violet or purple color developed	Presence of triterpenoids
Test solution + 2ml of Fehling's reagent + 3ml of water	Red-orange color formed	Presence of reducing sugars
Test solution + very small quantity of anthrone + few drops of concentrated Sulphuric acid and heat	Green to purple color developed	Presence of sugars
Test solution taken with 2 N HCl. Aqeous layer formed decanted add to which one or few drop of Mayer's reagent was added.	White precipitate or turbidity formed	Presence of alkaloids
Test solution in alcohol + one drop of neutral ferric chloride(5%) solution	Intense color developed	Presence of phenolics
Test solution in alcohol + Erlich reagent and few drops of concentrated HCl	Pink color formed	Presence of catachins
Test solution in alcohol + a bit of magnesium and one or two drops of concentrated HCL and heat	Red or orange red color formed	Presence of flavanoids
Test solution + water and shake	Foamy lather formed	Presence of saponins
Test solution + water + lead acetate	White precipitate developed	Presence of tannins
Test solution + magnesium acetate solution	Pink color developed	Presence of anthroquinones

Phytochemistry

The results of the phytochemical analysis are shown in Table 2. The chemical constituents of the extract include steroids, terpenoids, glycosides and saponins together with trace amounts of alkaloids and tannins. The results infer that roots carry all the identified

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components in considerable quantities than the other parts.

Table 2. HPLC analysis technical specifications

Compounds	Column	Mobile phase	Flow rate	Detector UV
Flavanoids	C_{18}	Water/methanol(80:20)	1ml/min	280nm
Alkaloids	C_{18}	Acetonitrile/water(50:50)	1ml/min	240nm

Table 3. Qualitative phytochemical analysis if various parts of Vanilla planifolia

Plant constituents	Plant part used for extraction	
	Leaf	Stem
Steroids	2+	3+
Terpenoids	3+	-
Alkaloids	2+	2+
Catachins	-	-
Flavanoids	1+	1+
Saponins	-	-
Tanins	-	-
Anthraquinon glycosides	2+	-

3+*: Appreciable amount, 2+: Moderate amount, 1+: Trace amount, -: Complete absence*

HPLC analysis of Vanilla planifolia leaf extract

The HPLC analysis of the plant extract showed the peaks indicating 18 alkaloids and 11 flavanoids. However, further studies are required to identify the specific alkaloids and flavanoids (Fig. 11 & 12)

Conclusion

Vanilla planifolia appears to have broad spectrum of antimicrobial action as evidenced from this study. The result of the various screening tests also indicate that all the parts of this plant, *viz*., leaves, and stem possess some measurable inhibitory action against the pathogens studied. It is of interest to note that *Pseudomonas aeruginosa*, one of the very resistant bacteria to synthetic drugs, was also found to be very susceptible to the leaf extract of this plant. Hence, this plant can be a potential source for evolving newer antimicrobial compounds.

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