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DETERMINATION OF THE PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL ACTIVITY OF *ACORUS CALAMUS*

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ABSTRACT
Ethanol extracts of *Acorus calamus* rhizome was used traditionally in India for the treatment of many diseases. The present study was investigated for *invitro* antimicrobial activity against pathogens namely *Staphylococcus aureus, Bacillus subtilis, Streptococcus pyogenes, Pseudomonas auroginosa, Klebsiella pnemonia, Aspergillus níger, Trichoderma viride* and *Candida albicans* using the well diffusion method. The results relevant that the rhizome extract possessed the highest inhibitory activity against both bacteria (*Staphylococcus aureus* in 20 mm) and fungi (*Candida albicans* in 12 mm). In parallel study was performed to identify the distribution and the concentration of the active compounds in rhizome of this plant. For this purpose we have prepared alcoholic extracts from each part of the plant and we have studied them separately.

KEYWORDS: Antimicrobial activity, *Acorus calamus*, Phytochemical analysis.

INTRODUCTION
Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many of them based on their use in traditional medicine. Various medicinal plants have been used for daily life to treat disease all over the world. They have been used as a source of medicine. The widespread use of herbal remedies and healthcare preparations, such as those described in ancient texts like the Vedas and the Bible has been traced to the occurrence of natural products with medicinal properties. In fact plants produce a diverse range of bioactive molecules making them a rich source of different types of medicines. Higher plants as sources of medicinal compounds have continued to play a dominant role in the maintenance of human health since ancient times. Over 50% of all modern clinical drugs are of natural product origin and natural products play an important role in drug development programs in the pharmaceutical industry (Boominathan and Ramamurthy, 2009).

There has been a revival of interest in herbal medicines. This is due to increased awareness of the limited ability of synthetic pharmaceutical products to control major diseases and the need to discover new molecular structures as lead compounds from the plant kingdom. Plants are the basic source of knowledge in modern medicine. The basic molecular and active structures for synthetic fields are provided by rich natural sources. The worldwide interest in medicinal plants reflects recognition of the validity of many traditional claims regarding the value of natural products in health care.

Acorus calamus Linn. commonly known as Sweet Flag, belongs to the family Araceae (Adoraceae). It is also called as *Acorus odoratus*. The genus Acorus derived from Acoron (coreon = the pupil of the eye) and the species calamus is derived from the Greek word Calamos (a reed). The family Araceae comprises about 110 genera and more than 1,800 species. The members of the family are rhizomatous or tuberous herbs. *Acorus calamus* commercially occurs in both peeled and unpeeled forms. This perennial herb is common on the banks of streams and in damp marshy places. The sweet flag oil present in this plant is a unique source of oxygenated sesquiterpenes of great structural variety (Ramamurthy and Sagaya Giri 2013). Apart from this terpenes a few commonly occurring steroids and xanthones had also been reported. The rhizome of the plant has medicinal properties against bugs, moths, lice, emetic stomach in dyspepsia; etc (Renu Rai *et al*. 1999). Common names of *Acorus calamus* which are used in different parts in India are Bach (Hindi), Vashampu (Tamil), Baje (Kannada) and Vasa (Telugu). Previously isolated classes of compounds from the plant material are hydroxyanthraquinones, glycosides, chrysophanic acid, kampferin and sannoxide A and B (Abo *et al*., 1998; Kochar, 1981). Against this background information and appreciating the knowledge of medicinal plants an effect

has been made in this study to evaluate the antimicrobial efficacy of *Acorus calamus* medicinal plants and also characterizing them by screening preliminary by phytochemical analysis. The study also pertains to inculcate the subject about the utilization of natural flora as therapeutic agents.

MATERIALS AND METHODS

Plant material and oil distillation

The medicinal herb of *Acorus calamus* rhizomes were purchased from herbal store at Pattukkottai, Thanjavur district, Tamil Nadu, South India. The rhizomes were identified with the help of flora of presidency, Tamil Nadu and Karnatic flora (Gamble, 1967; Matthew, 1983) and standard references (Krtikar and Basu, 1935).

The fresh rhizome of *Acorus calamus* was collected and a part of the rhizomes material was washed under running tap water. Small hairs of *A. calamus* were removed and could be chopped and dried at low temperature and then homogenized to fine powder and stored in airtight bottles. Powder of rhizome extracted with 90% w/w ethanol using a soxhlet apparatus. The ethanol was removed under pressure using a rotary evaporator. The dried residue of the crude extract was stored in a dark bottle at 4°C in airtight bottles for further studies. Approximately, 5 g of extract was obtained from 100 g of dried powder material. The extracts were dried in an air conditioned room at 25°C, milled and submitted to hydro distillation in a Clevenger-type apparatus for 4 hours. The extracts were dried in anhydrous sodium sulphate, filtered, stored in amber glass bottles in a refrigerator (4°C) for investigation of chemical constituents and antimicrobial activity.

Phytochemical Analysis

The preliminary phytochemical evaluation of rhizome was carried on extract prepared by successive extraction method in Soxhlet. The previously dried powdered (50 gm) were extracted in a Soxhlet apparatus with ethanol successively. The resultant extracts were evaporated to dryness under vacuum. These extract were subjected to chemical test for different phytoconstituents viz. alkaloids, carbohydrates, phenolics, flavonoids, proteins, amino acids, saponins, mucilage and resins etc.

Chemical tests were carried out on the ethanol and aqueous extracts using procedures to identify the phytochemicals as described by Sofowara (1993), Trease and Evans (1983), and Harborne (1973). Alkaloids, carbohydrates, tannins and phenols, flavonoides, gums and mucilage, fixed oils and fats and saponins were qualitatively analyzed.

Antimicrobial Assay

The following organisms were employed for this study as test organisms: Bacteria such as *Staphylococcus aureus, Bacillus subtilis, Streptococcus pyogenes, Pseudomonas auroginosa* and *Klebsiella pnemonia.* Fungi such as *Aspergillus níger, Trichoderma viride* and *Candida albicans*. The test microbial pathogen cultures were obtained from the stock cultures maintained in specific agar medium.

Antibacterial and antifungal activity of above mentioned extracts were tested using the agar diffusion method described by Collins and Lyne (1970). All the above-mentioned bacteria were inoculated into nutrient agar medium and fungi inoculated to potato dextrose agar medium. Wells of 6 mm diameter were punctured in the culture medium using sterile cork borer. Different extracts were administered to fullness in each well. Culture plates were incubated at 37^{o}C for 24 h in bacteria and incubated at 37^{o}C for 4 days in fungi. Bioactivity was determined by measuring diameter of inhibition zones in mm. Solvents used for extraction served as control.

RESULTS AND DISCUSSION

The result of phytochemical screening of the aqueous and alcoholic extracts of *Acorus calamus* revealed that the presence of alkaloids, flavanoids, phytosterols, tannins and phenols (Table 1). The plant extract of *Acorus calamus* used for the present work was choosing on the basis of their medicinal values. The natural plant parts are having a wide range of medicinal properties like antimicrobial, diuretic, emollient, febrifuge, narcotic, purgative and sedative. Previous study in the naturally the ethanolic extracts of *Acorus calamus* were subjected for phytochemical analysis. Phytochemical screening of the crude extract revealed that the presence of alkaloids, cardiac glycosides, terpenoids, saponins, tannin, flavonoids and steriods, but reducing sugars, carbonyl (aldehyde) and Phlobatanin show negative results (Makinde *et al*., 2007).

This plants growing under natural conditions contain the spectrum of secondary metabolites such as phenols, flavanoids, quinones, coumarins, tannins and their glycosides, alkaloids, essential oils etc., the importance of these substance as microbial agents against the pathogen has been emphasized by several workers (Sofowara, 1993). In the present study, it was clearly understood that the alcohol extracted maximum amount of the different type of metabolites present in the *Acorus calamus*. Boominathan and Ramamurthy (2009) reported that the phytochemical analysis of the *H. indicum* and *C. procumbens* extracts showed the presence of tannins, alkaloids, flavonoids and phenolic compounds. Tannins have been found to form irreversible complexes with proline-rich proteins.

Ethanolic extracts were tested against bacteria and fungi. Among the extracts, the rhizome extract of *Acorus calamus* were effective against bacteria and fungi. The antibacterial activity crude extract is shown in Table 2. The extracts showed maximum activity against *Staphylococcus aureus, Streptococcus pyogens, Klebsiella pnemonia* and *Pseudomonas aurogonosa*. These data revealed that rhizome extracts of *Acorus*

calamus exhibited significant antimicrobial activity. In testing, inhibition zone increased with increase in drug concentrations and thus exhibiting concentration dependent activity. The plants are the vital source of innumerable number of antimicrobial compounds. Several phytoconstituents like flavanoids (Tsuchiya *et al.*, 1996), phenolics and polyphenols (Mason and Wasserman, 1987), tannins (Ya *et al.*, 1988), terpenoids (Scortichini and Pia Rossi, 1991), sesquiterpenes (Goren, 1996) etc., are effective antimicrobial substances against a wide range of microorganisms.

The extracts showed maximum activity against *E. coli*, *Enterobacter aerogenes* and *Alcaligenes faecalis*. These data revealed that extracts of *R. tetraphylla* exhibited significant antibacterial activity (Suresh *et al.*, 2008). Apart from antimicrobial activity exhibited by tannins, they also react with proteins to provide the typical tanning effect. Medicinally, this is important for the treatment of inflamed or ulcerated tissues (Mota *et al.*, 1985). Tannins have important roles such as stable and potent antioxidants (Trease and Evans, 1983). Herbs that have tannins as their main component are astringent in nature and used for treating intestinal disorders such as diarrhoea and dysentery, thus exhibiting antimicrobial activity. One of the largest groups of chemical produced by plant is the alkaloids and their amazing effect on humans has led to the development of powerful pain killer medications (Raffauf, 1996).

H. indicum and *C. procumbens* are used for the treatment of inflammation, wound healing, antitumor and antianelgesic, hence different formulations could be prepared for clinical trials (Boominathan and Ramamurthy, 2009). It is hoped that this study would lead to the establishment of some compounds that could be used to formulate new and more potent antimicrobial drugs of natural origin. Studies are in progress to further evaluate the mechanisms of action *Acorus calamus* extracts on some organisms associated with human diseases. Hence, the present study suggests that pathogenic microorganisms may become resistant to existing drugs. Moreover, this study shows that some plants show much promise in the development of phyto-medicines having antimicrobial properties. In this endeavour, traditional herbal medicines must perforce be granted the benefits of modern science and technology to serve further global needs. The drugs derived from herbs may have the possibility of use in medicine because of their antibacterial activity.

Table 1: Qualitative Phytochemical screening on extracts of *Acorus calamus*

S. No	Name of Test	Test applied / Reagent used	Rhizome extract
1	Alkaloids	A] Mayer's B] Wagner's C] Hagner's D] Dragndorff's test	+ + + +
2	Flavanoids	HCl and magnesium turnings	+
3	Carbohydrate	Molisch's test	+
4	Tannins & Phenols	A] 10% Lead acetate B] $Fecl_3$	+ +
5	Test for Steroids	A] Salkowski's Test B] Libermann-Burchard's Test	+ +
6	Gums & Mucilages	Alcoholic Precipitation	-
7	Fixed oil & Fats	Spot test	+
8	Saponins	Foam test	-
9	Phytosterols	LB test	+
10	Volatile oils	Hydro distillation method	+
11	Protein & free amino acids.	A] Biuret test B] Ninhydrin test C] Xanthoprotein test	+ + +

-, absents; +, present.

Table 2. Antimicrobial efficacy of *Acorus calamus*

S. No	Organism	Zone of inhibition in mm
	Bacterial species	**Rhizome extract**
1	*Staphylococcus aureus*	20
2	*Bacillus subtilis*	10
3	*Streptococcus pyogens*	19
4	*Pseudomonas aurogonosa*	17
5	*Klebsiella pnemonia*	18
	Fungal species	
6	*Aspergillus niger*	11

7	*Trichoderma viride*	07
8	*Candida albicans*	12

REFERENCES

1. Abo KA, Adediwura AA, Ibikunle AJ. 1998. 1st International Workshop on Herbal Medicinal Products, University of Ibadan, Ibadan, Nigeria. pp. 22 - 24.
2. Boominathan, M and Ramamurthy, V. 2009. Antimicrobial activity of *Heliotropium indicum* and *Coldenia procumbens*. *J. Ecobiol.*, 24(1): 11 – 15.
3. Collins, CH and Lyne, PM. 1970. Mirobiological methods. 3rd ed. Butterworth and Co. Ltd. 414-427.
4. Gample RD. 1967. Chemical examination of the leaves of *Diospyros peregrina* Gurke. Indian Journal of Chemistry, 2: 129-130.
5. Goren, N., Woerdenbag, H and Bozok-Johansson, C. 1996. Cytotoxic and antibacterial activities of sesquiterpene lactones isolated from *Tanacetum praeteritum* subsp. *praeteritum*. *Planta Medica*. 62: 419-422.
6. Kochar SL. 1981. Tropical Crops: A Textbook of Economic Botany. London: McMillan, International College Editions, p. 416.
7. Kirthikar KR, Basu BD. 1935. Indian Medicinal Plants, vol. III. Periodical Experts, New Delhi, p. 1596–1598.
8. Makinde A. A., J. O. Igoli, L. TA'Ama, S. J. Shaibu, A. Garba. 2007. Antimicrobial activity of *Cassia alata*. Afr. J. Biotechnology. 6(13): 1509-1510.
9. Mason, T.L and Wasserman, BP. 1987. Inactivation of red beet betaglucan synthase by native and oxidized phenolic compounds. *Phytochemistry.* 26: 2197-2202.
10. Matthew, KM. 1983. The Flora of the Tamil Nadu Carnatic. The Rapinat Herbarium, St Joseph's College, Tiruchirapalli, India.
11. Mota, MLR., Thomas, G and Barbosa Filho, JM. 1985. Anti-inflammatory actions of tannins isolated from the bark of *Anacardium occidentale* L. *J. Ethanopharmacol.* 13: 289–300.
12. Raffauf, RF. 1996. A Guide to Their Discovery and Distribution, Hawkworth Press Inc, New York. p.35.
13. Ramamurthy, V and Sagaya Giri R. 2013. Hepatoprotective Activity of *Acorus calamus* Linn in Paracetamol intoxicated Albino Rats. Inter. J. Pharmacol. Drug Res., 2(1): 11–18.
14. Renu Rai, Aditi Gupta, Siddiqui, IR and Singh, J. 199. Indian Journal of Chemi., 38B: 1143-1144.
15. Scortichini, M and Pia Rossi, M. 1991. Preliminary *in vitro* evaluation of the antimicrobial activity of terpenes and terpenoids towards *Erwinia amylovora* (Burrill). *J. Applied Bacteriol.*, 71: 109-112.
16. Sofowora EA. 1993. Medicinal Plants and Traditional Medicine in African, John Wiley and Sons Ltd, Nigeria, p. 1-3.
17. Suresh, K., Saravana Babu, S and Harisaranraj, R. 2008. Studies on *Invitro* Antimicrobial Activity of Ethanol Extract of *Rauvolfia tetraphylla*, *Ethnobotanical Leaflets*, 12: 586-590.
18. Trease, GE and Evans, WC. 1983. Text book of Pharmacognosy. 12th ed. Balliere, Tindall, London, p. 57-59.
19. Tsuchiya, H., Sato, M., Miyazaki, T., Fujiwara, S., Tanigaki, S., Ohyama, M., Tanaka, T. and Iinuma, M. 1996. Comparative study on the antibacterial activity of phytochemical flavanones against methicillinresistant *Staphylococcus aureus*. *Journal of Ethnopharmacol.*, 50: 27-34.
20. Ya, C., Gaffney, S.H., Lilley, T.H and Haslam, E. 1988. Carbohydrate-polyphenol complexation. p. 553. In: Hemingway, R.W. and Karchesy, J.J. (ed.), *Chemistry and significance of condensed tannins.* Plenum Press; New York.