Int. J. Chem. Sci.: 7(3), 2009, 1905-1910

PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL INVESTIGATION OF *TYPHA ANGUSTIFOLIA LINN*

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ABSTRACT

Typha angustifolia of the family typhaceae is commonly known as elephant grass. It is mainly used in folk remedies for the treatment of tumours, as anticoagulant, astringent, sedative and tonics. The present study was to isolate and characterise phytochemical constituents and to find out the anti-microbial activity of the extract and isolated compounds of *Typha angustifolia*. The powdered material was defatted with petroleum ether followed by the extraction of mark by using Soxhlet extraction process. The extract was dried and concentrated and was used for further investigation. Dried extract showed the presence of alkaloids, sterols and flavanoids. Then the extract was subjected to isolation of chemical substance by column chromatography. Two compounds were isolated from methanolic extract and these were characterised by IR, MS and spectro polarimeter. The characterisation report showed the presence of nonacosanol and lupeol acetate. The methanolic extracts and the isolated compounds were subjected to the antimicrobial activity against both bacteria and fungi like *Aspergillus flavus*, *Serratia*, *E. coli*, *Listeria* and *Staphylococcus aureus* by disc diffusion method. It was compared with standard drug (ciprofloxacin, griseofulvin). Methanolic extract and isolated compounds were found to possess potent antibacterial and antifungal activity.

Key words : *Typha angustifolia,* Antimicrobial activity, Nonacosanol, Lupeol acetate

INTRODUCTION

Plants have been one of the main sources of medicaments either in the form of traditional preparations or pure active principles since the dawn of the human civilisation. The utilisation of plant based drugs in the world is flourishing and ever increasing. India has a wealthy ancestry of science on plant based drugs both for use in remedial and preventive medicine. Charaka Samhita reports the use of nearly 2000 vegetable remedies

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against various diseases. Nearly 60% of all pharmaceuticals are plant based[1]. More than 300000 plants have already been explored for their medicinal properties.

Typha angustifolia of the family typhaceae[2] is known as elephant grass and it is mainly used in folk remedies for the treatment of tumour, anticoagulant, astringent, sedative, tonic[3] etc. As literature survey revealed that not much work has been reported on *Typha angustifolia*, in the present study, the leaf portion of the plant was investigated for its pharmacognostical properties. Preliminary photochemical screening has been carried out followed by the isolation and characterization of chemical constituents and the antibacterial and antifungal activity of the various extracts as well as the isolated compounds were examined by disc diffusion method against microbes. In the present study, an endeavour has been made to find out the antibacterial and antifungal activity of various extracts obtained from the plant.

EXPERIMENTAL

Materials and method

Collection of plant material

The dried flowers and leaves of *Typha angustifolia* were collected from Watrap (Tamilnadu) and authenticated by CCRAS, Bangalore. The whole plant was processed and stored in airtight container for further use.

Pharmacognostical evaluation

Typha angustifolia was studied for its quantitative standards like moisture content, total ash, extractive values and its microscopical studies on leaves and powder characteristics were also conducted on the arial parts of the plant.

Extraction and phytochemical screening

The dried plant materials were defatted with petroleum ether and extracted with methanol by using Soxhlet extraction process.

The methanolic extract of *Typha angustifolia* was subjected to various phytochemical analysis[4].

Separation techniques

Optimisation of TLC solvent system

Different solvent systems were taken for developing the TLC system[5] for identification of various constituents present in the methanolic extract and the solvent showing maximum separation was selected as mobile phase for the study. The following solvents were used for the development of TLC in different ratios-toluene : ethyl acetate, chloroform : methanol, ethyl acetate : petroleum ether, petroleum ether : benzene, benzene : chloroform, chloroform : acetone, n-butanol and acetic acid : water.

Isolation of phytoconstituents by column chromatography

Methanolic extract was subjected to column chromatography[6] by successive methods based on the TLC patterns and photochemical screening. The methanolic extract was chromatographed on a silica gel column (60-120 mesh Merck) and eluted with chloroform : acetone (9 : 1). A few fractions obtained were combined, concentrated and it was separated with chloroform, , which gave a single spot in the above-mentioned solvent system.

Characterisation of isolated compounds

The isolated compounds were characterised by IR and MS spectral data.

Anti microbial activity of the extract and the isolated compounds

Antibacterial and anti fungal activities of different extract and isolated compounds were tested by the disc- diffusion method

Preparation of extracts

The plant part was powdered and extracted successively with different solvents according to the polarity of the solvents (non-polar to polar). Extraction was done by successive Soxhlet extraction method. The plant extract was filtered through Whatman filter paper No. 1 into a beaker. The filtrates were dried until constant dry weights of each extracts were obtained. The residues were stored at 4ºC for further use.

Microorganisms used

Bacteria - *Staphylococcus aureus*, *Escherichia coli*, *Serratia*, *Listeria*. and fungus; *Aspergillus flavus*.

Preparation of inoculums

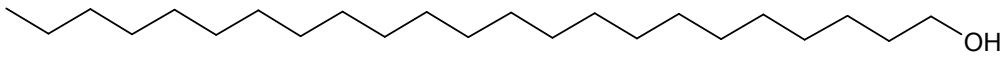
Stock cultures were maintained on slopes of nutrient agar at 4ºC. Active cultures for the experiments were prepared by transferring a loopful of cells from the stock cultures to the test tubes of Mueller-Hinton broth (MHB) for bacteria and Sabouraud Dextrose broth (SDB) for fungi that were incubated without agitation for 24 hrs at 37ºC and 25ºC, respectively. The cultures were diluted with fresh SDB and MHB.

Antimicrobial susceptibility test

The disc- diffusion method was performed to evaluate the antimicrobial activity. *In vitro* anti microbial activity was screened using Mueller Hinton Agar (MHA) obtained from Himedia (Mumbai). The MHA plates were prepared by pouring 1.5 mL of molten media into sterile plates. The plates were allowed to solidify for 5 minutes and 0.1 % inoculum suspension was swabbed uniformly and the inoculum was allowed to dry for 5 minutes. The different extracts were loaded on 3 mm sterile disc till saturation. The loaded disc was placed on the surface of the medium and the compound was allowed to diffuse for 5 minutes. The plates were kept for incubation at 37º C for 24 hrs. At the end of incubation; the inhibition zones formed around the discs were measured with transparent ruler in millimetre. Using Sabouraud dextrose agar plates for fungus also followed the same procedure. These studies were performed in triplicate by using standard drugs (10 mcg/disc penicillin; for bacteria and 20 mcg / disc for fungi).

RESULTS AND DISCUSSION

Typha angustifolia leaves were subjected to qualitative standards like moisture content, total ash, acid insoluble ash, extractable value (Table 1). Microscopical studies on the TS of leaf and powder was done. (Number of stomata-5, stomata index-8.4%, number of palisade cells-28, palisade ratio-7). The preliminary photochemical screening of the methanolic extract of the leaves of the plant showed the presence of alkaloids, sterols and flavanoids. IR and MS were recorded to characterise the isolated compounds. These data confirmed the compounds as nonacosanol and lupeol acetate. From the mass spectral data, the molecular formula of nonacosanol has been found to be $C_{18}H_{38}O$ and the melting point of the isolated compound was found to be 65ºC. The IR spectrum confirms the presence of a hydroxyl group in nonacosanol by exhibiting an absorption band at 3420 cm^{-1}. The terminal methylene group attached to the functional group OH was found to be at 2926.28 cm^{-1} while terminal methyl group was found to be at 1410 cm^{-1}.

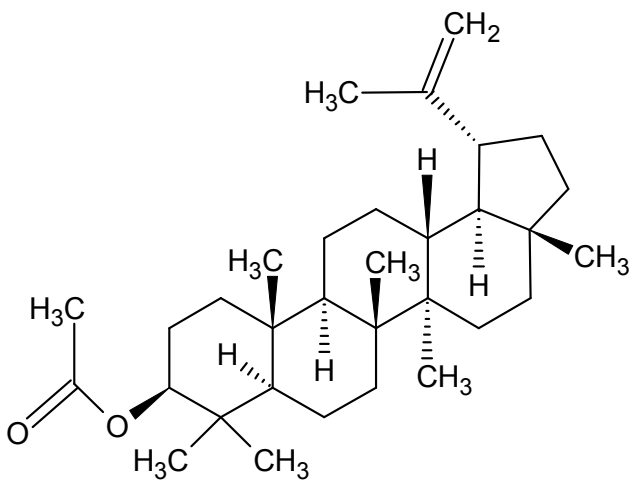


Nonacosanol

The molecular formula of lupeol acetate was found to be $C_{32}H_{52}O$ and the structure was confirmed by its mass spectrum , which indicate a molecular ion peak at m/z = 427. The melting point of the isolated compound was found to be 216ºC, which very well matched with that of lupeol acetate. The optical rotation of the particular compound was determined by spectro polarimeter and it was found to be $[\alpha]_{D26.6}°$. Its IR spectrum also showed the presence of an ester carbonyl group at 1736 cm^{-1} and exomethylene group at 2851, 1660 and 719 cm^{-1}.

Table 1 : Qualitative analysis

Parameters (%)	Inference
Loss on drying	8.21
Total ash	11.15
Acid insoluble ash	0.54
Water soluble extractive value	16.8
Alcohol soluble extractive value	33.6



Lupeol acetate

In this antimicrobial study, the methanolic extract and isolated compound exhibited significant activity against *E. coli* and moderate activity against *Staphulococcus aureus*

when compared with the standard drug. The methanolic extract exhibited moderate activity against *Aspergillus flavus.* Nonacosanol possesses a significant activity against *Serratia*. The result showed that methanolic extract of *Typha angustifolia* have potent antifungal and antibacterial activities.

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Accepted : 07.04.2009