STRUCTURAL ISOLATION OF NEROLIDOL THROUGH NMR STUDIES FROM ALPINIA CALCARATA ROSCOE (ZINGIBERACEAE) - A VALUABLE MEDICINAL PLANT

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ABSTRACT
Plant products are a part of phyto medicine since time immemorial. The characteristic property of medicinal plants is due to a variety of complex chemical compounds and hence these plants are generally referred to as ‘natural bio-chemical factories’ or ‘chemical goldmines’.

The current study aimed at isolation of Nerolidol, a valuable compound from Alpinia calcarata. Thin Layer Chromatography, High Performance Liquid Chromatography, Column Chromatography, Nuclear Magnetic Resonance were used in this process.


INTRODUCTION
Medicinal plants are one of the essential sources of phytochemicals to combat new strains of microorganisms. Extraction and characterization of several active phytocompounds from plants have given birth to some high activity profile drugs.[1] The use of traditional medicine is widespread in India.[2]

Natural products from medicinal plants, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug leads because of the unmatched chemical diversity.[3] Natural products and secondary metabolites formed by living systems, notably from plant origin, have shown great potential in treating human diseases such as cancer, coronary heart diseases, diabetes and infectious diseases.[4]
Plant products can be derived from any part of the plant body like bark, leaves, flowers, roots, fruits, seeds etc.\textsuperscript{[5]} Phytochemical studies have played a significant role in giving the solution to systematic problems on the one hand and in the search for additional resources of raw materials for pharmaceutical industry on the other.\textsuperscript{[6]}

Knowledge of the phytochemical constituents of plants is necessary for the discovery of therapeutic agents. Validated knowledge is also a valuable lead for disclosing new sources of other useful compounds such as tannins, oils, gums, flavanoids, saponins, essential oils precursors for the synthesis of complex chemical substances.\textsuperscript{[7]}

Several species of \textit{Alpinia} have been reported to contain several type of flavanoids including chalcones, flavanones, proanthocyanidin, flavonols and flavones,\textsuperscript{[8]} sesquiterpenes,\textsuperscript{[9]} labdane diterpenes\textsuperscript{[10]} diarylheptanoids\textsuperscript{[11]} and kava pyrone\textsuperscript{[12]}. Drugs from the rhizomes of \textit{Alpinia calcarata} are used in treatment of rheumatism, bronchial catarrh and asthma.

**MATERIALS AND METHODS**

The rhizomes were washed in running water, sliced into small pieces and then shade dried for a week at 35-40\(^\circ\)C. They were pulverized in an electric grinder and exhaustively extracted successively in a soxhlet extractor with petroleum ether, acetone, methanol and water by hot percolation for 3 days. After completion of extraction the dark brown extract was then cooled, filtered, concentrated using rotary evaporator and finally by vacuum suction to get a crude dried extract and yield was calculated, stored in sterile container for further use.

**Separation of components by thin layer chromatography**

For the present study, clean glass plates of 10x5cm size were carefully washed with methanol : water, 9:1(v/ v), then dried at ambient temperature for 3 h. TLC was performed on commercial glass plates precoated with 0.25mm layers of silica gel F\textsubscript{254} (Merck, ± 1.05715). The plates were activated at 120\(^\circ\)C for 30 min and then used. On a coated TLC plate the solution of the extracts (5\(\mu\)l) was applied as a thin spot. Chromatograms were run in small glass tanks linked with chromatography paper equilibrated with the running solvent (Hexane: Ethyl acetate). The resolution bands were obtained and retardation factor (Rf) values were calculated.
Separation by Column chromatography

Column chromatography was carried out on silica gels 60-120 mesh glass column was used as a purification technique. The plant residue (about 2.5gm) was subjected to a gradient elution silica gel column chromatography (CC) using different solvents. Finally, the mobile phase consisting of Hexane: EtOAc (100:0 – 0:100) was optimized and eluted almost fifteen fractions (AC1-AC15).

Each fraction was subjected to TLC, the fractions of similar TLC patterns were combined, concentrated, re chromatographed over silica gel columns of 60cm x 3cm to isolates active compound and confirmed by qualitative analysis. Spots and bands of compounds on TLC were detected using UV light.

Nuclear Magnetic Resonance (NMR) spectral analysis

Proton NMR spectroscopy (1H-NMR) and Carbon 13 NMR (13C-NMR) spectroscopy were used for the NMR studies. In 1H-NMR, the solvent CDCl3 was used with tetramethyl silane (TMS) as an internal standard and the chemical shifts are given in δ- values. The 13C spectrum offers further characterisation of the molecule as it relates directly to the carbon skeleton. It provides information on the types of carbon atoms present, the number of distinct kinds of carbon atoms present equals the number of peaks in proton decoupled spectra.

HPLC analysis

After phytochemical analysis HPLC analysis for the isolated compound 1 of Alpinia calcarata was performed on Shimadzu LC 10AVP HPLC system equipped with a binary pump SPD 10 AVP pump.

In HPLC 10μl of the filtered sample were injected to the automatic injector using a microsyringe (1-20μl, Shimadzu). The analysis was done on LC column with a reverse phase C-18- Phenomenex and column dimension of 5cm x 1.5 cm. The mobile phase was 2% THF in water : 1% THF in Acetonitrile (40:60) in an isocratic method. The sample was eluted at a flow rate of 2.0ml/min with a column temperature maintained at 25±2°C at 254nm.
RESULTS AND DISCUSSION

The retention time of the isolated compound AC.1 (nerolidol) was 2.50/Min and peak area obtained under the curve was 132 (Fig.1).

NMR Spectroscopy
The $^1$H NMR and $^{13}$C NMR were recorded using a Bruker spectrophotometer (400 MHz, CDCl$_3$).

Fig.1. HPLC chromatogram

![HPLC Chromatogram](image1)

Fig.2. $^1$H NMR Spectral Analysis

![$^1$H NMR Spectral Analysis](image2)
Signals at $\delta$ 0.0-2.5 in the H-NMR spectrum of the compound were signals due to the Alkane groups [-CH$_3$-](Fig.2). The spectrum contained signals at 2.5-5.0 showed one shift due to the presence of an [OH] group. In the H-NMR spectrum of the compound is without any aromatic rings at 5.0-7.0, but signals at 7.0-10.0 showed 2 shifts for OH vibrations. In $^{13}$C-NMR (400MHz,CDCl$_3$) of the compound AC1(fig.3) showed signals at 0-50 showed 6 shifts corresponding to C-H saturated alkanes, but signals at 50-100 showed 5 shifts corresponding to C-OH groups. In $^{13}$C-NMR data of the compound, signals 100-200 did not confirm any aromatic carbons.

Fig.3.$^{13}$C NMR Spectral Analysis

Structure of the isolated compound: Nerolidol
Table 1. Structural details of Nerolidol

<table>
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<tr>
<th>IUPAC Name</th>
<th>(6E)-3,7,11-trimethyldodeca – 1,6,10-trien-3-ol</th>
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<td>Molecular Formula</td>
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<td>Parachor</td>
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CONCLUSION

The results obtained in this study thus suggest the identified phytochemical compounds may be the bioactive constituents and this plant is proving to be an increasingly valuable reservoir of bioactive compounds of substantial medicinal merit. Therefore, the data generated from these experiments have provided the chemical basis for the wide use of *Alpinia calcarata* as therapeutic agent for treating various ailments.

REFERENCES


