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Isolation and Characterization of a Novel Chemical Entity from Ether Extract of *Ammomum subulatum* Leaves

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ABSTRACT

Recently there has been a shift in universal trend from synthetic to herbal medicine, which we can say 'Return to Nature'. *Ammomum subulatum* which is commonly known as large cardamom is belonging to family Zingiberaceae. Plant has plenty of medicinal values. This paper represents the isolation of a new chemical entity, cardamonin, present in the leaves of *Ammomum subulatum* and their medicinal aspects. Cardamonin is a well known compound previously isolated from seeds of *Ammomum subulatum*, having antimicrobial activity. This time leaves of *Ammomum subulatum* was used as a plant material due to large availability in nature than seeds, for isolation of biological active compounds. Extraction of chlorophyll free dried leaves was done with petroleum ether. Concentrate the extract and then isolate the compounds column chromatography using silica gel as an absorbent. Diethyl ether extract of the drug was poured in the silica column and then eluted successively with different solvents; in the increasing order of polarity Different fractions of compounds were separated and marked. The fractions having same Rf values were combined together and concentrated. Concentrated fractions were crystallized with ethyl acetate. Identification of the isolated compound was confirmed by physico-chemical data, spectral interpretation and elemental analysis.

KEY WORDS: *Ammomum subulatum*, extraction, isolation.

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INTRODUCTION

The frequency of life-threatening infections caused by pathogenic micro-organisms has increased worldwide and is becoming an important cause of morbidity and mortality in immune compromised patients in developing countries.[1] In recent years, attempts have been made to investigate the indigenous drugs against infectious diseases. This may help to develop safer antimicrobial drugs.[2] *Ammomum subulatum* (Large Cardamom) is belongs to family Zingiberaceae, is commonly known as 'Bari Ilaichi', has been reported to possess antimicrobial, antioxidant, antidiabetic and antihelicobector activity etc.[3-8] *Ammomum subulatum* has traditionally been used to GIT disorders.[9]

It is an important economic crop in the Eastern Himalayas and typically cultivated in woodland areas with overhead shade and access to regular irrigation from mountain streams. This species is native to the eastern Himalayas; the production regions are Nepal and Sikkim, a tiny Indian union state located between Nepal and Bhutan. It grows vigorously during the summer monsoon months.[10]Most of the work has been carried out time to time on seeds of the plat by various scientists but no such great work was found on leaves of the *Ammomum subulatum*, even plant is rarely ever wholly leafless during the year. The various chemical constituents isolated from plant parts of A*mmomum subulatum* are glycosides, petunidin 3,5-diglucoside, leucocyanidin-3-O-β-D-glucopyranoside, cholcone, flavanone, alpinetin and subulin. Acid hydrolysis of subulin gave the aglycone, subulaurone. The seeds on steam distillation yield a dark brown, mobile essential oil (2.5%) having a characteristic odor of cineol. Volatile oils present in seed containing cineol (74%), limonene (10.3%), myrcene (0.3%), α-terpinene (0.2% and 4-terpinene (0.2%).[11]

MATERIALS AND METHODS

The plant material (Leaves) of *Ammomum subulatum* was collected from Palampur, Himachal Pradesh, India in the month of October 2010. It was identified by NISCAIR, New Delhi, Ref. no. NISCAIR/RHMD/Consult/2010-11/1547/145. Leaves of *Ammomum subulatum* were dried in shade and reduced to coarse powder for extraction, isolation and characterization of chemical constituents.

Extraction

Leaves of A*mmomum subulatum* were coarsely powdered in a suitable grinder. These powdered leaves were poured in glass container and sufficient quantity of acetone solution was added in this container. Stir the solution until a nice dark green liquid was achieved then separate the green colored chlorophyll-acetone solution from coarse powdered leaves using separating funnel.[12-13] 5 kg chlorophyll free

powdered leaves of *ammomum subulatum* were extracted with 10 liter of petroleum ether in a Soxhlet apparatus for 48 hr. After complete extraction, the extract was concentrated using a rotary evaporator to afford a yellowish mass.[13] Prepared yellowish mass was then subjected to isolation of different compound followed by various spectral analytical techniques for the identification of the individual compounds.

Isolation of compound

Silica gel (60-120 mesh) was used as absorbent for column chromatography. The column was taken and packed with glass wool at the bottom of the column. The slurry was prepared using silica gel and hexane. It was poured slowly from the top of the column in a little quantity allowing for the uniform packing. 2/3rd of the column was packed by using above procedure. The remaining completely dried silica gel slurry containing the diethyl ether extract of the drug was poured in the silica column (mesh size 60-120, 50 × 12 cm) and then eluted successively with different solvents, in the increasing order of polarity like n-hexane, petroleum ether (40-60°), Benzene : acetone (9:1), Benzene : acetone (8:2), Benzene : acetone (7.5:2.5), Benzene : acetone (5:5), Benzene : acetone (4.5:5.5) and ethanol. The fractions were collected and marked. The marked fractions were subjected to thin layer chromatography to check homogeneity of various fractions. The fractions having same Rf values were combined together and concentrated.[14] Crystallize the selected concentrate with ethyl acetate and yielded the compound.

Identification of the isolated individual compounds

An IR spectrum of compound was recorded on FTIR system by using potassium bromide pellets.

^{1}HNMR spectra of the compounds were recorded on NMR spectrophotometer in DMSO using TMS as internal standard.

For Mass spectra compound was dissolved in dissolving solvent and then diluted with water containing 0.1% formic acid and 80% acetonitrile before injection into the MS system mass charge was adjusted at some values.[15]

RESULT AND DISCUSSION

A new compound was isolated first time from ether extract of *Ammomum subulatum* leaves was obtained as yellow prism crystals with 0.1% yield, showed violet color on TLC plate under UV chamber had following properties-

Rf value: 0.4 [Chloroform: methanol (10:1 v/v)], **Empirical formula:** $C_{16}H_{14}O_4$, **m.pt.:** 208-209°C

UV: λmax(DMSO): 210 nm [Ethanol: water (5:5 v/v)];

FTIR (KBr): 3174.6 (O-H), 3060.4 (Ar C-H stretching), 2947.3, 2836.4 (Methyl C-H stretching), 1716.5, 1620.0 (C=O stretching), 1580.0, 1558.5 (C⋯C ring stretching), 1358.2 (O-H bending), 1188.2, 1114.2 (Alcoholic C-O stretching) and 946.8 cm^{-1} (Trans H-C=C-H).;

^{1}H-NMR : δ 3.84 (s, 3H, OCH_3), 4.54 (s, 1H, Aromatic OH), 4.55 (s, 1H, Aromatic OH), 5.85 (s, 1H, ArH), 5.93 (s, 1H, ArH), 7.25-7.43 (m, 5H, ArH), 7.59 (d, 1H, trans olefinic), 7.84 ppm (d, 1H, trans olefinic).;

ESI full mass-MS: m/z 269 $[M-H^+]$, 253 $[M-OH]^+$, 193 $[M-C_6H_5]^+$, 131, 103, 77.

Elemental Analysis: Calculated for $C_{15}H_{11}O_3$: C, 71.10; H, 5.22; O, 23.68.

Found: C, 71.12; H, 5.23; O, 23.65.

On the basis of the spectroscopic evidence, isolated compound was established as cardamonin has following structure. [Figure 1].

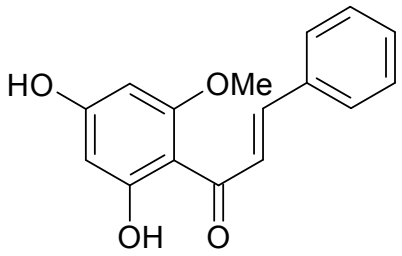

Figure 1: Cardamonin

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