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Phytochemical screening of Rhizome extract of *Curcuma zedoaria* (Christm) Roscoe by HRLC-MS technique

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ABSTRACT

Phytochemical investigation of methanol extracts of *Curcuma zedoaria* (Christm) Roscoe rhizome (Zingiberaceae) yielded 9 major phytoconstituents by using High Resolution Liquid Chromatography - Mass Spectroscopy (HRLC-MS) technique. The mass spectra of compounds found in extract was matched with Metlin database library, results confirmed the presence of therapeutically potent chemical compounds. Metabolites analysis by ESI-Q-TOF-MS revealed presence of 9 major abundant compounds namely Glycopyrrolate, Cucurbitacin I, Flurandrenolide, 26,26,26,27,27,27-hexafluoro-1alpha, 24-dihydroxyvitaminD3 / 26,26,26,27,27,27 - hexafluoro-1alpha, Proto porphyrinogen IX, Phenylbutazone glucuronide, Methyl Gamboginate, Propofol, Ibuprofen. This report is the first of its kind to analyze chemical constituents of *Curcuma zedoaria* using HR-MS. In addition to this, the results of HRMS profile can be used as pharmacognostical tool for identification of plant.

Key words: *Curcuma zedoaria,* Phytochemical, HRLC-MS, Cucurbitacin I, Flurandrenolide.

INTRODUCTION

Curcuma zedoaria (Christm) Roscoe belongs to Zingiberaceae, and commonly known as 'Zedoary', 'White turmeric' (English), 'Jangli haldi' (Hindi), 'Shati' (Sanskrit), 'Kachora' (Kannada), 'Karppurakkilangku' (Tamil), 'Meitei yaingang' (Manipuri), 'Aam aadaa' (Bengali).The essential oils of *C. zedoaria* possess the efficient cyto-toxic effects on non-small cell lung carcinoma cells (NSCLC) and causes apoptosis *in vitro* and *in vivo* (Chen *et al.,* 2013). (Tholkappiyavathi, *et al,* 2013) Reviewed the analgesic activity, anti-inflammatory, anti-hyperlipidemic, anti-arthritic, anticancer, antidiabetic activity, anti-oxidant and *In-vitro* antibacterial activities of *C. zedoaria.* Curdione a chemical compound obtained from *C. zedoaria* significantly suppress tumour growth in a xenograft nude mouse breast tumour (MCF-7

cells) in a dose dependant manner and inhibits cell proliferation and induced cell apoptosis (Li, *et al*., 2014). Petroleum ether extracts of *C. zedoaria* inhibits the proliferation of human triple negative breast cancer cell line MDA-MB- 231 (Gao *et al*., 2014). Active compound isocurcumenol isolated from the rhizomes of *C. zedoaria* inhibits the proliferation of cancer cells without inducing the significant toxicity to normal cells and *in vivo* doses of 37.7 mg/kg body weight significantly reduce the ascetic tumor in DLA-challenged mice and increase the life span compare to untreated control mice (Lakshmi, 2011).

Herbs, root tubers many; sessile tubers small, cylindrical, branched 2-2.5 x 1-1.5cm long white inside. Rhizome, pale yellow, bluish at center and white towards periphery, 2.5-5 x 2-2.7 cm. Leafy shoot 2-3 feet tall or more in length; leaves 4-6, 25-65 x 10-15cm long, oblong lanciolate with acuminate apex, narrowed to the base, purple colored strip on the entire midrib on upper surface of the leaves; petiole shorter than leaf lamina. Inflorescence central, 10-15 x 3-4 cm; spike 10-15 x 2-3.5 cm; flowers yellow shorter than bracts, 2.5-3.5cm; comma bract dark pink or shade of others 4.5-5.0 x 1-2 cm; fertile bract ovate, green, slightly twinged with red 3-4 x 1.5-2 cm; calyx obtusely toothed, whitish; corolla tube funnel shape, 3 cm long; capsule 3-gonous, smooth, thin, ovoid, dehiscing irregularly; seeds oblong or ellipsoid, with lanceolate, white aril.

MATERIAL METHODS

The plant material of *Curcuma zedoaria* (Christm) Roscoe was collected from Amgoan, district Gondia of Maharashtra and identified by using authentic floristic literature (Sharma, *et al.,* 1996; Pradhan, *et al*., 2005). The voucher herbarium specimen (ASJ 7624) is deposited in VH Herbarium, department of Botany, Vivekanand Arts, Sardar Dalipsingh Commerce and Science College, Samarth Nagar, Aurangabad.

Extract preparation

The collected rhizomes was washed with water and shade dried, rhizome powder was prepared with the help of mortal and pestle and extracted through Soxhlet using methanol as solvent and heated at 65^0C for 18-24 hours, extract was kept for evaporation and sample was stored in amber coloured bottle for further phytochemical screening which was carried out using

Fig. 1: HRLC-MS spectrum of *Curcuma zedoaria* rhizome extract

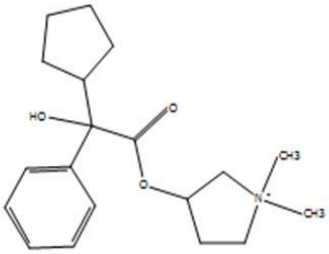
HRLC- MS technique.

Instruments and chromatographic conditions

Equipment and conditions for identification of metabolites from an active sub-fraction of methanol extract was carried out at SAIF, IIT Bombay. Samples were analyzed on a LC- ESI-Q-TOF-MS (Agilent Technologies 6550 i-Funnel) system equipped with a G4220B pump, G4226A auto sampler and G1316C, and a diode array detector (DAD). The elution solvent consisted of a gradient system of 0.1% formic acid in water (A) and acetonitrile (B) at a flow rate of 0.3 ml/min. The gradient system started with 95% A: 5% B reaching 5% A: 95% B in 50 min., then back to initial composition 95% A: 5% B in 10 min which was held at same composition for 5 min. The MS analysis was carried out by ESI positive ionization mode. MS source conditions were as follows: capillary voltage 3500 V, Gas temperature 250 C, drying gas flow 13 L/min, sheath Gas temp 300, sheath Gas Flow 11, nebulizing gas pressure 35 (psig), fragmentor 175 V, Skimmer 65 V, Octopole RF Peak 750 V, and mass range m/z 50–1000. The resolution was 40,000 FWHM. Metlin database was used to structure confirmation.

RESULTS & DISCUSSION

In the present study we characterized chemical profile of *Curcuma zedoaria* (Christm) Roscoe using HRMS spectra. The chromatogram showed relative concentrations of various compounds getting eluted as a function of retention time. The heights of peak indicate relative concentrations of components present in plant extract. The mass spectrometer analyzes compounds eluted at different times to identify nature and structure of the compounds. The large compound fragments into small compounds giving rise to appearance of peaks at different m/z ratios. These mass spectra are fingerprint of that compound which can be identified from the data library. Compound prediction is based on Dr. Duke's Phytochemical and Ethnobotanical Databases.



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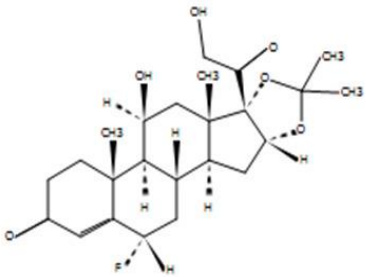

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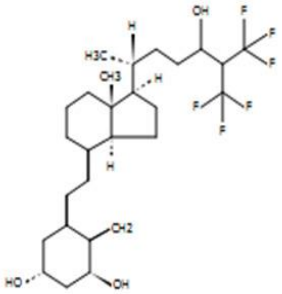

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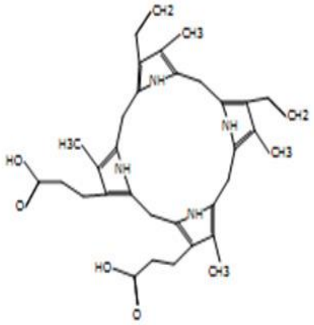

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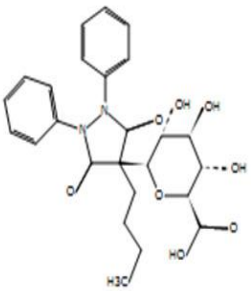

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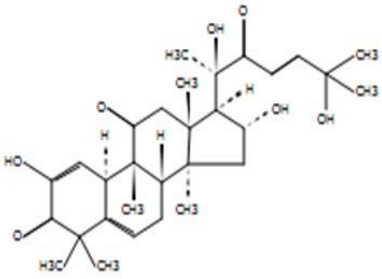

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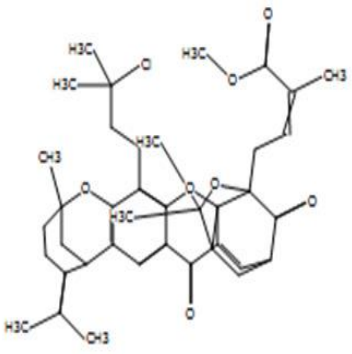

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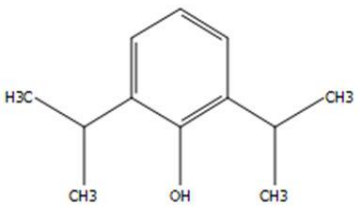

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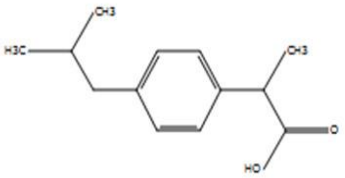

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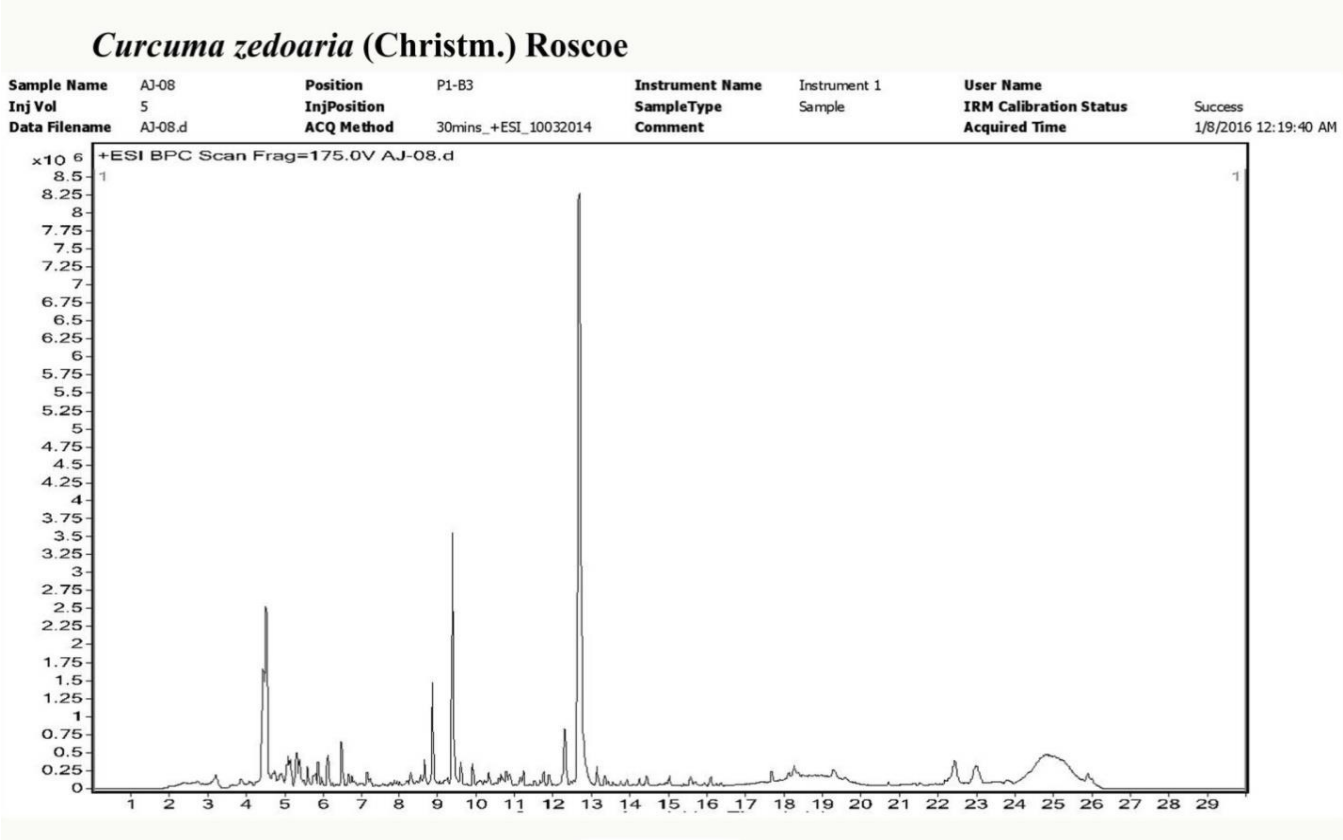

Table A: Major abundant metabolites from *Curcuma zedoaria* (Christm) Roscoe rhizome

Sr. no	Name of Compound	RT	Mass	Formula	M/Z
1	Glycopyrrolate	5.094	318.2051	$C_{19}H_{28}NO_3$	318.2047
2	flurandrenolide	5.147	436.2256	$C_{24}H_{33}F_6O_3$	437.2327
3	26,26,26,27,27,27-hexafluoro-1alpha,24-dihydroxyvitaminD3	5.616	524.2758	$C_{27}H_{38}F_6O_3$	525.2834
4	Protoporphyrinogen IX	5.809	568.3017	$C_{34}H_{40}N_4O_4$	569.3093
5	Phenylbutazone glucuronide	6.137	484.1885	$C_{25}H_{28}N_2O_8$	485.1956
6	Cucurbitacin I	8.858	514.2882	$C_{30}H_{42}O_7$	537.2373
7	Methyl Gamboginate	9.59	662.3011	$C_{39}H_{47}ClO_7$	663.3081
8	Propofol	12.67	178.1368	$C_{12}H_{18}O$	201.1261
9	Ibuprofen	12.671	206.1317	$C_{13}H_{18}O_2$	229.1209

Results revealed that presence of major abundant metabolites identified in *Curcuma zedoaria* (Christm) Roscoe methanolic rhizome extract fraction by ESI-QTOF-MS analysis were Glycopyrrolate, Flurandrenolide, 26,26,26,27,27,27-hexafluoro-1alpha, 24- dihydroxyvitaminD3 / 26,26,26,27,27,27 - hexafluoro- 1alpha, Proto porphyrinogen IX, Phenylbutazone glucuronide, Cucurbitacin I, Methyl Gamboginate, Propofol, Ibuprofen (spectra 1-9). The retention time, m/z value, mass, molecular formula and the DB difference (ppm) of the major 9 abundant metabolites are shown in table, the spectra showed counts versus mass to charge (m/z) ratio (Table 1).

CONCLUSION

The results revealed that presence of major abundant metabolites identified in the *Curcuma zedoaria* (Christm) Roscoe methanolic rhizome extract fraction by ESI-QTOF-MS analysis were Glycopyrrolate, Flurandrenolide, 26,26,26,27,27,27-hexafluoro-1alpha,24-dihydroxyvitaminD3 / 26,26,26,27,27,27 -hexafluoro-1alpha, Proto porphyrinogen IX, Phenylbutazone glucuronide, Cucurbitacin I, Methyl Gamboginate, Propofol, Ibuprofen. HRMS analysis showed the existence of various compounds with different chemical structures. The presence of various bioactive compounds confirms the application of *Curcuma zedoaria* (Christm) Roscoe for various ailments by traditional practitioners. However, isolation of individual phytochemical constituents may proceed to find a novel drug.

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Conflicts of interest: The authors stated that no conflicts of interest.

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