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PHYTOCHEMICAL PROFILE OF *CURCUMA INODORA* Blatt. RHIZOME EXTRACT

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ABSTRACT

Curcuma inodora Blatt. (Zingiberaceae), rhizome used in the treatment of Psychosomatic disorders, constipation, in muscular pain, also have anti-inflammatory, antipyretic and wound healing activity. Methanolic rhizome extracts screened for detection of phytochemical constituents by using HRLC-MS technique.Metabolites analysis by ESI-Q-TOF-MS revealed presence of 14 major compounds namely 1alpha,25-dihydroxy-26,27-dimethyl-20,21,22,22,23,23-hexadehydro-24a- homovitamin D3, Trp Gln Trp, N-(1-methyl-2-hydroxy-2-phenyl-ethyl) arachidonyl amine, Arg Lys Ile, Methylprednisolone succinate, (6R)-vitaminD3 6,19-(4-phenyl-1,2,4-triazoline-3,5-dione)adduct, Prednicarbate, N-Palmitoyl-L-serine phosphoric acid, 1-(9Z,12Z,15Z-octadecatrienoyl)-2- (4Z,7Z,10Z,13Z,16Z,19Z-docosahexaenoyl)-sn-glycerol, benzonatate(2,5,8,11,14,17,20,23,26- Nona oxaoctacosan-28-ylp-(butylamino)benzoate, (17E)-1alpha,25-dihydroxy- 26,27-dimethyl - 17, 20,22,22,23,23-hexade hydro -24a- homovitamin D3, Salmeterol, 1,2-ditetradecanoyl-sn- glycero-3-phospho-(1'-sn-glycerol) and Digitoxin. This report is the first of its kind to analyze chemical constituents of *Curcuma inodora* using HR-MS. In addition to this, results of HRMS profile can be used as pharmacognosticaltool for identification of *Curcuma* species.

Key words: *Curcuma inodora*, Phytochemical,HRLC-MS

INTRODUCTION

*C. inodora*Blatt commonly known a 'Scentless turmeric', 'hidden lily'.Tribal peoples of Nandurbar district in Maharashtra use small pieces of rhizomes of *C. inodora* soaked in a cup of water for 4-5 hours and decantation administered once a day for 2-3 days in the treatment of Psychosomatic disorders, constipation and in the treatment of muscular pain **(Patil** and **Bhaskar, 2006).** The methanol extract of rhizome showed the anti-inflammatory, antipyretic and wound healing activity at doses of 200 mg/kg of body weight **(Bharati *et al.*, 2009).**

Herbs, root tuber numerous oblong, ellipsoid, white inside 2-3.5×1-1.5cm. Rhizome small, conical, pale yellow inside having no odour, 2-3 × 2-2.5 cm. Leafy shoot 20-30cm tall; leaves radical, 5-7, petiolate, broadly elliptic, lanciolate, tip acuminate, plicate; lamina 10-15× 5-7.5cm long, winged, deeply concave. Inflorescence central; peduncle 5-7 cm long; Spike 5-10 × 2.5-3cm long; flower 2-3×1-1.5cm; coma bracts violet, tip rounded, 3-3.5×1-1.5cm long; calyx 3 lobed at apex, 1-1.2 cm white; corolla purpulish with a yellow streak on the lip; corolla tube 1.5-2cm; anther thecae parallel, white having pink base, spur bent inwards; stigma bilipped; style long, filiform; ovary trilocular with many ovules; seeds many, ovoid, arilate, brown, 2.5-3mm.

MATERIAL AND METHODS

Plant material of *C. inodora*Blatt.was collected from Ajara town, Kolhapur of Maharashtra state and identified by using floristic literature (**Sharma,*et al.*, 1996; Sabu, 2006;Yadav and Sardesai, 2002**). The voucher herbarium specimen (ASJ 7622) deposited in VH Herbarium, department of Botany, Vivekanand Arts, Sardar Dalipsingh Commerce and Science College, Samarth Nagar, Aurangabad, Maharashtra.

Preparation of crude extracts

The dried rhizome powder was extracted through Soxhlet using methanol as solvent and heated at 65^0C for 18-24 hours, extract was kept for evaporation and the sample was stored in amber colored bottle and further, the phytochemical analysis was carried out using HRLC- MS technique **(Jadhao and Bhuktar, 2017).**

HRLC- MS analysis

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, Powai, 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 AND DISCUSSION

The results pertaining to HRLC-MS analysis led to the identification of number of compounds from the HR fractions of the methanol extract of *C. inodora* Blatt. These compounds were identified through mass spectrometry attached with HRLC; the results of the present study were tabulated in (**Table 1**). Metabolites analysis by ESI-Q-TOF-MS revealed the presence of 14 major abundant metabolites identified in methanol rhizome extract fraction of *C. inodora* Blatt were1alpha,25-dihydroxy-26,27-dimethyl-20,21,22,22,23,23-hexadehydro-24a- homovitamin D3, Trp Gln Trp, N-(1-methyl-2-hydroxy-2-phenyl-ethyl) arachidonyl amine, Arg Lys Ile,

Methylprednisolone succinate, (6R)-vitaminD3 6,19-(4-phenyl-1,2,4-triazoline-3,5-dione)adduct, Prednicarbate, N-Palmitoyl-L-serine phosphoric acid, 1-(9Z,12Z,15Z-octadecatrienoyl)-2- (4Z,7Z,10Z,13Z,16Z,19Z- docosahexaenoyl)-sn-glycerol, benzonatate(2,5,8,11,14,17,20,23,26- Nona oxaoctacosan-28-ylp-(butylamino)benzoate, (17E)-1alpha,25-dihydroxy- 26,27-dimethyl - 17, 20,22,22,23,23-hexade hydro -24a- homovitamin D3, Salmeterol, 1,2-ditetradecanoyl-sn- glycero-3-phospho-(1'-sn- glycerol) and Digitoxin (Spectra 1-14). The retention time, m/z value, mass, molecular formula and the DB difference (ppm) of the major 10 abundant metabolites are shown in (Table 1), the spectra showed counts versus mass to charge (m/z) ratio **(fig. 1).**

Table 1: Phytochemicals identified in methanol rhizome extracts of *C. inodora* Blatt

Sr. no	Name of compound	RT	Mass	Formula	M/Z
1	1alpha,25-dihydroxy-26,27-dimethyl-20,21,22,22,23,23-hexadehydro-24a-homovitamin D3	5.889	452.337	$C_{30}H_{44}O_3$	475.3233
2	Trp Gln Trp	7.23	518.2311	$C_{27}H_{30}N_6O_5$	519.2384
3	N-(1-methyl-2-hydroxy-2-phenyl-ethyl) arachidonyl amine	9.78	437.3364	$C_{29}H_{43}NO_2$	438.3439
4	Arg Lys Ile	9.956	415.295	$C_{18}H_{37}N_7O_4$	438.2844
5	Methylprednisolone succinate	12.402	474.2231	$C_{26}H_{34}O_8$	497.2124
6	(6R)-vitaminD3 6,19-(4-phenyl-1,2,4-triazoline-3,5-dione)adduct/(6R)-cholecalciferol 6,19-(4-phe	12.951	559.3674	$C_{35}H_{49}N_3O_3$	560.3753
7	Prednicarbate	13.391	488.2382	$C_{27}H_{36}O_8$	511.2276
8	N-Palmitoyl-L-serine phosphoric acid	13.559	423.2363	$C_{19}H_{38}NO_7P$	424.2437
9	1-(9Z,12Z,15Z-octadecatrienoyl)-2-(4Z,7Z,10Z,13Z,16Z,19Z-docosahexaenoyl)-sn-glycerol	14.101	662.4764	$C_{43}H_{66}O_5$	663.4845
10	benzonatate(2,5,8,11,14,17,20,23,26-Nona oxaoctacosan-28-ylp-(butylamino)benzoate	14.236	603.3698	$C_{30}H_{53}NO_{11}$	604.3777
11	(17E)-1alpha,25-dihydroxy- 26,27-dimethyl - 17, 20,22,22,23,23-hexade	14.236	452.3331	$C_{30}H_{44}O_3$	453.3406

	hydro -24a- homovitamin D3				
12	Salmeterol	15.407	415.2701	$C_{25}H_{37}NO_4$	438.2595
13	itetradecanoyl-sn- glycero-3-phospho- - glycerol)	16.012	667.4578	$C_{34}H_{68}O_{10}P$	668.4645
14	Digitoxin	17.702	764.4444	$C_{41}H_{64}O_{13}$	787.4339

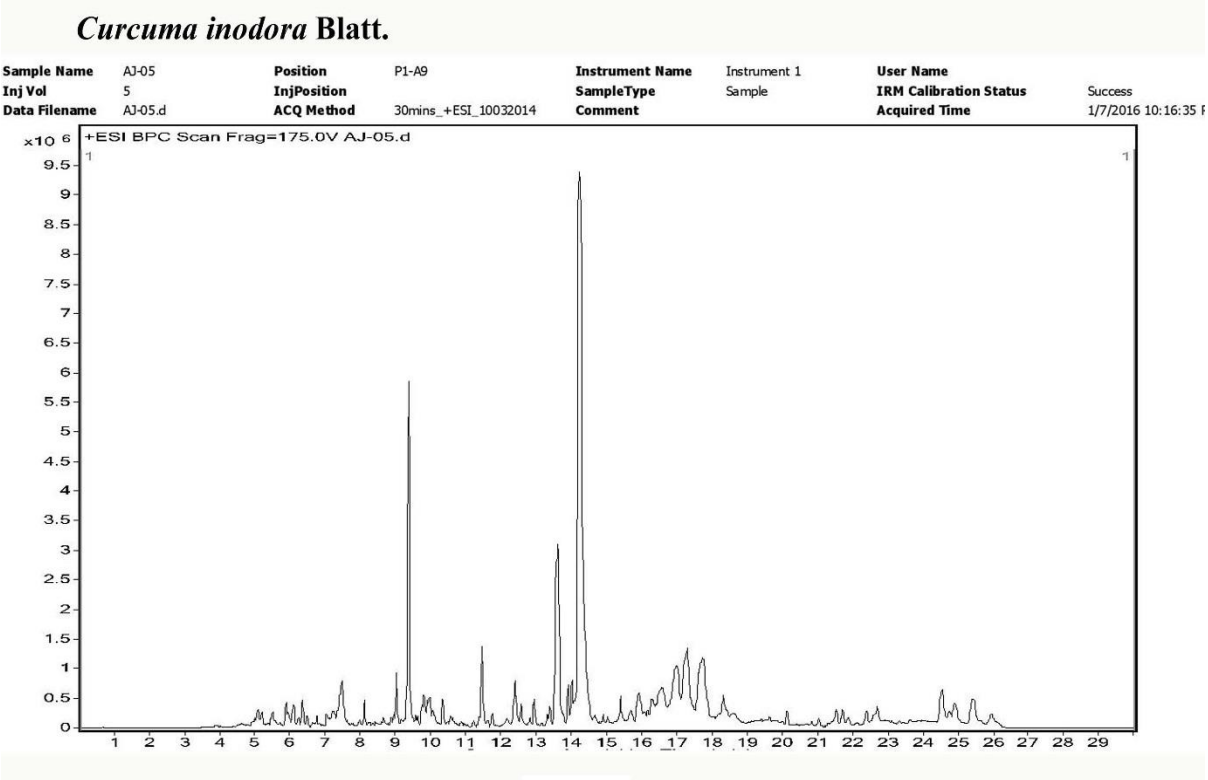

Fig. 1. HR-LCMS chromatogram of rhizome extract of *C. inodora*Blatt

In present study chemical profile of methanol rhizome extract of *C. inodora*Blatt using HRLC-MS spectra, chromatogram showed relative concentrations of various compounds getting eluted as a function of retention time. Hghts of peak indicate relative concentrations of components present in the plant. Mass spectrometer analyzes the compounds eluted at different times to identify nature and structure of compounds. Large compound fragments into small

compounds giving rise to appearance of peaks at different m/z ratios (**fig. 1**). These mass spectra are fingerprint of that compound which can be identified from the data library. In addition to this, the results of HRMS profile can be used as pharmacognostical tool for the identification of the plant.

CONCLUSION

The presence of various bioactive compounds i.e. 1alpha,25-dihydroxy-26,27-dimethyl-20,21,22,22,23,23-hexadehydro-24a- homovitamin D3, Trp Gln Trp, N-(1-methyl-2-hydroxy-2-phenyl-ethyl) arachidonyl amine, Arg Lys Ile, Methylprednisolone succinate, (6R)-vitaminD3 6,19-(4-phenyl-1,2,4-triazoline-3,5-dione)adduct, Prednicarbate, N-Palmitoyl-L-serine phosphoric acid, 1-(9Z,12Z,15Z-octadecatrienoyl)-2- (4Z,7Z,10Z,13Z,16Z,19Z-docosahexaenoyl)-sn-glycerol, benzonatate(2,5,8,11,14,17,20,23,26- Nona oxaoctacosan-28-ylp-(butylamino)benzoate, (17E)-1alpha,25-dihydroxy- 26,27-dimethyl - 17, 20,22,22,23,23-hexade hydro -24a- homovitamin D3, Salmeterol, 1,2-ditetradecanoyl-sn- glycero-3-phospho-(1'-sn-glycerol) and Digitoxin. HRMS analysis showed the existence of various compounds with different chemical structures. The presence of various bioactive compounds confirms the application of *Curcuma inodora* Blatt for various ailments by traditional practitioners. However, isolation of individual phytochemical constituents may proceed to find a novel drug.

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