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HR-LCMS based phytochemical analysis of *Curcuma caesia* Roxb. rhizome

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ABSTRACT

Background: Medicinal plants have long been a prominent source of novel chemicals and bioactive compounds, owing to their rich history of use in traditional medicine. The extensive use of medicinal plant products in the pharmaceutical and biotechnology field has highlighted the need for detailed phytochemical profiling of these plants. **Objective:** To establish a comprehensive phytochemical profile of the Hydro-alcoholic (50% Ethanolic) extract of *Curcuma caesia* Roxb. rhizome using High Resolution-Liquid Chromatography Mass Spectrometry (HR-LCMS). **Materials and Methods:** The air-dried and powdered rhizome of *Curcuma caesia* Roxb. was extracted using a hydro-alcoholic solvent system (50% ethanol). The resultant extract was subjected to HR-LCMS for phytochemical analysis. **Result:** The HR-LCMS analysis of the hydro-alcoholic (50% ethanolic) extract of *Curcuma caesia* Roxb. rhizome identified a total of 25 compounds using both ESI positive and ESI negative modes, including several compounds with significant pharmaceutical value. **Conclusion:** The presence of various bioactive compounds justifies the traditional use of *Curcuma caesia* Roxb. rhizome in the treatment of various diseases and highlights its potential for further pharmaceutical exploration.

Keywords: Traditional medicine, *Curcuma caesia*, HR-LCMS, Bioactive compounds, Phytochemical profile.

INTRODUCTION

Medicinal plants are an inexhaustible reservoir of novel compounds that can be harnessed for drug development. They have played a significant role in traditional medicine since antiquity, providing remedies for various health conditions and promoting overall well-being [1,2]. The medicinal properties of herbs used in traditional medicine systems, such as Ayurveda and Traditional Chinese Medicine are related to the presence of various bioactive compounds [3,4]. Hence the study of a plant's medicinal potential begins with analyzing its chemical composition. The phytochemical data also plays a crucial role in the identification and authentication of the plant during traditional applications and research investigations. Additionally, this information helps in standardization of plant extracts used in scientific investigations [5,6].

With the emergence of modern hyphenated techniques, it has become possible to obtain detailed chemical profiles of herbal medicine preparations or extracts. Hyphenated analytical techniques like GC-MS and LC-MS are being used quite extensively for the analysis of components present in herbal medicine preparations and for quality assurance of these herbs. These methods have been extensively utilized in Traditional Chinese Medicine [3,4]. Crude natural product extracts, which are complex mixtures containing numerous compounds, can be effectively analyzed using suitable hyphenated techniques. Among these, HR-LCMS is one of the most commonly employed techniques for analyzing natural products. In HR-LCMS, if the ionization method is chosen appropriately it serves as a highly effective tool for screening crude plant extracts. This method facilitates the analysis of a wide range of compounds, from small non-polar molecules to large polar constituents such as oligosaccharides, proteins and tannins present in natural product extracts [3].

Curcuma caesia Roxb., commonly known as Black Turmeric, is a perennial herb with bluish-black rhizomes. It is predominantly found in Northeast and Central India. It is also sparsely distributed in the Papi Hills region of East Godavari, West Godavari and Khammam districts of Andhra Pradesh. The rhizomes hold significant economic value due to their reputed medicinal properties. They are traditionally used for various therapeutic purposes including smooth muscle relaxation, treatment of hemorrhoids, leprosy, asthma, cancer, epilepsy, fever, wounds, vomiting, menstrual disorders and inflammation. Additionally, they possess anthelmintic, aphrodisiac, and anti-gonorrheal properties. The aromatic rhizomes are bluish-black internally and have a distinctive sweet fragrance due to their essential oil content. In traditional medicine, the rhizomes are used to treat leucoderma, tumors, piles, bronchitis etc. It is also applied as a paste to treat bruises and rheumatic pain [7-10]. Despite its wide range of

traditional uses, *Curcuma caesia* Roxb. remains poorly documented in scientific literature. However, existing studies indicate that it exhibits antioxidant, anticancer, neuropharmacological, thrombolytic, anthelmintic, antiulcer and antidepressant activities [5].

The medicinal properties of *Curcuma caesia* Roxb. rhizome has been attributed to the presence of bioactive compounds [5,11]. Thus, the present study was carried out to detect the bioactive compounds present in the *Curcuma caesia* Roxb rhizome hydroalcoholic (50% ethanolic) extract using HR-LCMS (High Resolution - Liquid Chromatography and Mass Spectrometry). This study may provide an insight on its role in traditional medicine.

MATERIALS AND METHODS

Plant Materials- Collection and Authentication

Curcuma caesia Roxb. rhizome was collected from Bhugaon, Pune (Maharashtra) and authenticated from Plant Drug Authentication Service, Agharkar Research Institute, Pune, Maharashtra, India (Voucher specimen no. AUTH 22-125). Rhizomes were washed with running tap water to remove soil particles and air dried. After drying, it was ground into fine powder and stored in an airtight container at room temperature for further studies.

Preparation of Plant extracts

Extracts of *Curcuma caesia* Roxb. rhizome was prepared by refluxing 10 grams of air-dried powdered material in 100 ml of Hydroalcohol for 6 hours at 70 °C. The extracts were filtered through Whatmann filter paper no. 1. They were evaporated to dryness using a water bath at 80 °C. The extracts were reconstituted in HPLC grade Hydroalcohol at a concentration of 100 ppm and submitted for HR-LCMS analysis to Sophisticated Analytical Instrument Facility (SAIF), Indian Institute of Technology (IIT), Powai, Mumbai.

High resolution liquid chromatography and mass spectrometry (HR-LCMS) analysis

HR-LCMS studies and accurate mass measurements were carried out by 6550i funnel Q-TOF LCMS (Agilent Technologies, USA) equipped with a dual AJS ESI ion source. The stationary phase (Column) used was Hypersil GOLD C18 100 x 2.1mm, 3 microns (Agilent Technologies, USA) and the mobile phase used were

Solvent A: 0.1% formic acid in Milli-Q water
Solvent B: Acetonitrile

The data acquisition and processing were performed using Agilent Mass Hunter software. The mass spectral data was obtained in both ESI-positive and ESI-negative ionization modes. Mass spectra and distinctive mass fragmentation patterns were utilized to identify the compounds in the sample. The HR-LCMS mass spectrum was analysed by comparing the spectrum of unknown components with the spectrum of known components. The Metlin database has been utilized for comparison and identification of metabolites in this study. The components of the trial materials were identified by their names, molecular weights, and structures.

RESULT

LC-MS-based phytochemical profiling can be utilized to verify the authenticity of crude drugs available in the market [12]. In the present study the phytochemical profile of *Curcuma caesia* Roxb. rhizome extract was characterized by using High Resolution-Liquid Chromatography Mass Spectroscopy (HR-LCMS) spectra. The relative concentrations of different compounds get eluted as a function of retention time in a chromatogram (Figure 1 and 2). On the basis of the height of the peak, we could determine the relative concentration of bioactive compounds present in the extract. The mass spectrometer examines the compounds eluted at different times to determine the nature and structure of the compounds. These mass spectra can serve as fingerprints of that compound.

In the present study Phytochemical screening of Hydroalcoholic (50 % ethanolic) extract of *Curcuma caesia* Roxb. rhizome through HR-LCMS detected several phytocompounds. However, compounds having Hits (DB) 5 or less than 5 are selected as the most probable compounds present in the rhizome. Table 1 and 2 provide a summarized list of identified compounds, including their retention time, mass, molecular formula and hits (DB).

Major known compounds found to be present in *Curcuma caesia* Roxb. rhizome are Perphenazine, Metochlopramide, (3xi,6E)-1,7-Diphenyl-6-hepten-3-ol, 2-Undecyl-4(1H)-quinolinone N-oxide, Erythromycin ethylsuccinate, Proansamitocin, dolichyl phosphate, (all-E)-6'-Apo-y-caroten-6'-al, Thermozeaxanthin-15/ Zeaxanthin glucoside ester, Linamarin, Threoninyl-Lysine, Lilaline, L-Citronellol glucoside, Tangeraxanthin, Glimepiride, A-Nor-5alpha-cholestan-2-one, 5alpha-Tomatidan-3-one, (3S,3'S,5R,5'R,6R)-6,7-Didehydro-5,6-dihydro-3,3',5,8'-tetrahydroxy-beta-kappa-caroten-6'-one, Phylloquinone, Trimethobenzamide, Streptobiosamine, alpha-Zearalanol, 6-Hydroxypentadecanedioic acid, Sakacin A and 2,3,5-Trimethyl-6-[4-(methylthio)butyl]pyrazine.

Out of all the compounds Perphenazine, Metochlopramide, Erythromycin ethylsuccinate, Linamarin, Proansamitocin, Lilaline, L-Citronellol glucoside, Glimepiride, Phylloquinone, Trimethobenzamide, alpha-Zearalanol and Sakacin A possess various biological activities as given in Table 3.

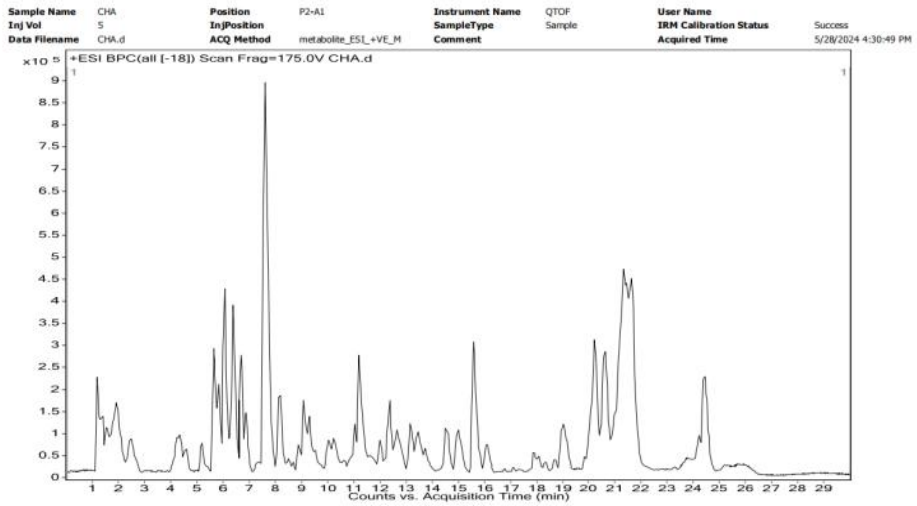

Figure 1: HR-LCMS Chromatogram of Hydroalcoholic extract of *Curcuma caesia* Roxb. Rhizome; ESI positive mode

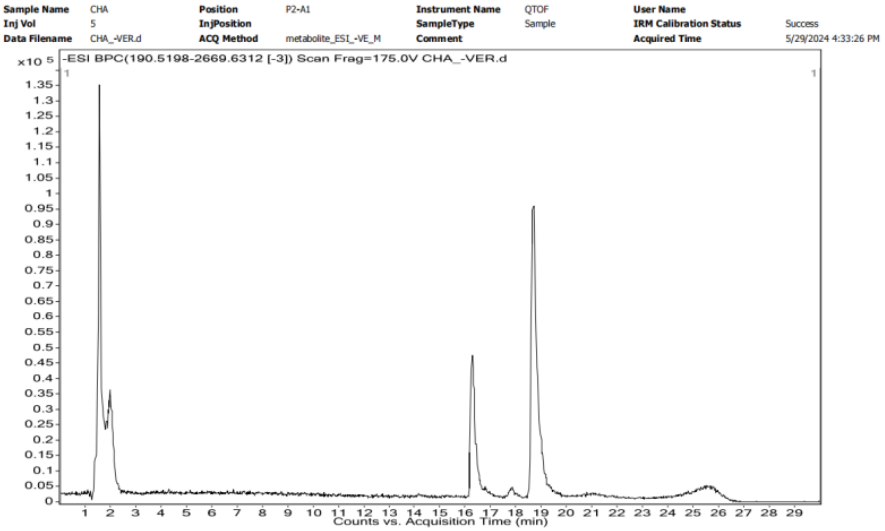

Figure 2: HR-LCMS Chromatogram of Hydroalcoholic extract of *Curcuma caesia* Roxb. Rhizome; ESI negative mode

Table 1: List of compounds identified in HR-LCMS (ESI positive mode) profiling of hydroalcoholic extract of *Curcuma caesia* Roxb. Rhizome

S. No.	Compound Name	Retention time (RT)	Mass	Formula	Hits (DB)
1.	Perphenazine	1.551	403.1542	C_{21} H_{26} Cl N_3 O S	1
2.	Metochlopramide	4.407	299.1434	C_{14} H_{22} Cl N_3 O_2	1
3.	(3xi,6E)-1,7-Diphenyl-6-hepten-3-ol	5.918	266.17	C_{19} H_{22} O	1
4.	2-Undecyl-4(1H)-quinolinone N-oxide	6.39	314.2157	C_{20} H_{28} N O_2	1
5.	Erythromycin ethylsuccinate	9.123	861.5012	C_{43} H_{75} N O_{16}	1
6.	Proansamitocin	9.563	443.2364	C_{25} H_{33} N O_6	1
7.	Dolichyl phosphate	20.574	440.3081	C_{25} H_{45} O_4 P	1
8.	(all-E)-6'-Apo-y-caroten-6'-al	21.362	442.3208	C_{32} H_{42} O	1
9.	Thermozeaxanthin-15/ Zeaxanthin glucoside ester	25.055	954.6894	C_{61} H_{94} O_8	1
10.	Linamarin	1.578	247.1019	C_{10} H_{17} N O_6	2
11.	Threoninyl-Lysine	4.12	247.1516	C_{10} H_{21} N_3 O_4	2
12.	Lilaline	4.284	383.1034	C_{20} H_{17} N O_7	2
13.	L-Citronellol glucoside	11.578	318.2019	C_{16} H_{30} O_6	2
14.	Tangeraxanthin	20.512	484.334	C_{34} H_{44} O_2	2
15.	Glimepiride	20.806	490.2298	C_{24} H_{34} N_4 O_5 S	2
16.	A-Nor-5alpha-cholestan-2-one	23.501	372.336	C_{26} H_{44} O	2
17.	5alpha-Tomatidan-3-one	9.186	413.3242	C_{27} H_{43} N O_2	3
18.	(3S,3'S,5R,5'R,6R)-6,7-Didehydro-5,6-dihydro-3,3',5,8'-tetrahydroxy-beta-kappa-caroten-6'-one	20.214	616.4105	C_{40} H_{56} O_5	3
19.	Phylloquinone	21.453	450.3495	C_{31} H_{46} O_2	3
20.	Trimethobenzamide	6.701	388.1973	C_{21} H_{28} N_2 O_5	4
21.	Streptobiosamine	8.504	337.1395	C_{13} H_{23} N O_9	4
22.	alpha-Zearalanol	11.503	322.1737	C_{18} H_{26} O_5	4
23.	6-Hydroxypentadecanedioic acid	14.893	288.1917	C_{15} H_{28} O_5	4
24.	Sakacin A	5.824	288.1784	C_{12} H_{24} N_4 O_4	5

Table 2: List of compounds identified in HR-LCMS (ESI negative mode) profiling of hydroalcoholic extract of *Curcuma caesia* Roxb. rhizome

S. No.	Name	Retention time (RT)	Mass (DB)	Formula	Hits (DB)
1.	2,3,5-Trimethyl-6-[4-(methylthio)butyl]pyrazine	14.082	224.1347	C_{12} H_{20} N_2 S	2

Table 3: Biological activities of some important bioactive compounds found in 50 % Hydroalcoholic extract of *Curcuma caesia* Roxb. Rhizome

S. No.	Compound Name	Biological activities
1.	Perphenazine	Anti-viral, Antipsychotic, Sedative and Antiemetic activity; Mainly used to treat Schizoprenia [13-22]
2.	Metochlopramide	Antiemetic activity, Beneficial in managing several gastrointestinal disorders [23-25]
3.	Erythromycin ethylsuccinate	Antibacterial activity [26]
4.	Linamarin	Anticancer activity [27-31]
5.	Proansamitocin	Used to produce a potent antitumor agent called ansamitocin [32]
6.	Lilaline	Potential anti-inflammatory properties [33-35]
7.	L-Citronellol glucoside	Positively correlated with gut bacteria [36]
8.	Glimepiride	Useful and cost-effective option for managing type 2 *Diabetes mellitus* [37]
9.	Phylloquinone	Major dietary source of Vitamin K1 [38]
10.	Trimethobenzamide	Antiemetic activity [39]
11.	alpha-Zearalanol	Prevents atherosclerosis [40]
12.	Sakacin A	Antilisterial activity, Antimicrobial activity [41]

DISCUSSION

Perphenazine, a piperazinyl phenothiazine drug marketed under the name Trilafon, has been used in clinical practice for decades. It is primarily prescribed to treat schizophrenia, particularly addressing symptoms such as hallucinations, delusions and auditory disturbances. Beyond its role in psychosis treatment, perphenazine has also been explored for treating malignant brain tumors. Additionally, studies have shown that perphenazine induces rapid apoptosis and inhibits the growth of T-cell acute leukemia (T-ALL) cells both *in vivo* and *in vitro*, demonstrating its antileukemic potential [13-17].

Interestingly, perphenazine also exhibits sedative and antiemetic activity [18-21]. Perphenazine also displays antiviral activity against RNA viruses by inhibiting clathrin-mediated endocytosis, virus-cell fusion, infection, replication, and host cell entry [22]. Notably, it exhibits antiviral properties against COVID-19. The drug interacts with the amino acid pocket site and disrupts the attachment of the virus, making it a promising candidate for combating COVID-19 infection [13,16].

The act of emesis is regulated by the vomiting center in the medulla, which integrates signals from the vestibular system, the chemoreceptor trigger zone (CTZ), the cortex, and the gut. Nausea and vomiting caused by certain cancer chemotherapy drugs are among the most distressing side effects of treatment. Antiemetics work by blocking different types of receptors located in various regions of the body [23]. Metochlopramide is one among the common antiemetic drugs used for preventing nausea and vomiting [23,24]. It is also beneficial in managing conditions such as esophageal reflux disease, gastroparesis, dyspepsia, and other functional gastrointestinal disorders. Additionally, it has been used to control vomiting related to narcotic analgesics, radiation therapy, pregnancy, gastroenteritis, gastric cancer, liver and biliary conditions, chronic kidney failure, heart disease, and alcoholism [25].

Erythromycin ethylsuccinate, the ethylsuccinate salt form of erythromycin, is a broad-spectrum topical antibiotic with antibacterial activity. It diffuses through the bacterial cell membrane and binds reversibly to the 50S ribosomal subunit, inhibiting bacterial protein synthesis. Depending on the organism's susceptibility, it can exhibit either bacteriostatic or bactericidal effects. Studies by Berube *et al.* (1988) revealed that erythromycin ethylsuccinate demonstrated slightly higher activity than erythromycin estolate against *Streptococcus pyogenes*, *Streptococcus pneumoniae*, and *Branhamella catarrhalis* with median MICs of less than 0.12 and 0.5 µg/ml, respectively [26].

Linamarin is a cyanogenic glycoside that possesses therapeutic properties and has been traditionally used to treat various diseases, including cancer. It is primarily extracted from *Sorghum* and *Cassava* species, respectively. The anti-cancer properties of the linamarin offer a promising and novel approach against cancer cells. Studies have demonstrated that the combination of linamarin and linamarase exhibits strong cytotoxic effects on MCF-7 and HT-29 cancer cell lines, as well as significant cytotoxicity against HeLa (cervical adenocarcinoma) and Caov-3 (ovarian) cancer cells, compared to linamarin alone [27-31].

Proansamitocin is used to produce a potent antitumor agent called ansamitocin which is a highly toxic maytansinoid [32]. Lilaline is a pyrrolidinoflavonol generally isolated from the aerial parts (flowers) *Lilium candidum* L. commonly called Madonna lily. It belongs to the family Liliaceae. It is believed that the flowers of *Lilium candidum* L. have anti-inflammatory properties according to traditional medicine [33-35]. L-Citronellol glucoside is a prenol lipid which is positively correlated with beneficial gut microbiota including *Flavonifractor*, *Oscillibacter* and *Ruminiclostridium* species. The gut microbiota or microorganisms residing in the gut are important for improved immunity, intestinal barrier, metabolism and host health [36].

Type 2 diabetes mellitus is marked by insulin resistance and the gradual decline in β cell function. Thus, β cell stimulators are crucial for achieving sufficient glycemic control. Glimepiride promotes insulin secretion from pancreatic β cells. It can be prescribed as monotherapy for patients whose blood sugar levels remain uncontrolled despite dietary and lifestyle changes or in combination with other antidiabetic medications like metformin and insulin when sulfonylureas alone are insufficient. The recommended dose ranges from 1 to 8 mg per day, though doses above 4 mg may not provide additional benefits. Caution is advised when prescribing glimepiride to elderly patients or those with kidney or liver disorders. Clinical research indicates that glimepiride causes fewer instances of hypoglycemia and less weight gain compared to other sulfonylureas. Moreover, it appears to be safer for patients with cardiovascular conditions. Glimepiride effectively lowers fasting plasma glucose, post-prandial glucose and glycosylated hemoglobin levels, making it an affordable and valuable option for managing type 2 diabetes [37].

Phylloquinone is a prenylated naphthoquinone. It is synthesized exclusively by plants, green algae, and certain cyanobacteria, where it functions as a key electron carrier in photosystem I. In humans and other vertebrates, phylloquinone plays the role of Vitamin K1. Vitamin K1 is an essential nutrient for blood clotting, as well as bone and vascular health in humans as well as other vertebrates. Green

leafy vegetables and vegetable oils are the major dietary sources of Vitamin K1 for humans [38]. Trimethobenzamide is primarily used as an antiemetic, particularly for managing nausea and vomiting caused by chemotherapy drugs [39]. alpha-Zearalanol is a phytoestrogen that prevents atherosclerosis [40]. Sakacin A is a 41-amino acid peptide with potent antilisterial activity, functioning by creating pores in the cell membrane. Its strong antimicrobial activity makes it a promising candidate for use as a biopreservative [41].

CONCLUSION

The HR-LCMS analysis revealed the presence of a wide range of bioactive components, which justifies the traditional use of *Curcuma caesia* Roxb. rhizome in the treatment of various diseases. However, isolation and identification of novel pharmacologically active compounds could pave the way for new research opportunities. The analysis detected a total of 25 compounds using both ESI positive and ESI negative modes. The major bioactive compounds with notable biological activities include Perphenazine, Metochlopramide, Erythromycin ethylsuccinate, Linamarin, Proansamitocin, Lilaline, L-Citronellol glucoside, Glimepiride, Phylloquinone, Trimethobenzamide, alpha-Zearalanol and Sakacin A. The diverse range of phytochemicals found in the rhizome of *Curcuma caesia* Roxb. can serve as a basis for standardization.

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Conflict of interest

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