International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 19-07-2021, Accepted: 04-08-2021, Published: 19-08-2021
Volume 6, Issue 4, 2021, Page No. 787-791

A study of preliminary and GC-MS analysis of *Curcuma pseudomontana* J. Graham

T Parvathi[1], M Sathish[1], P K Siva[1], R Bhuvaneswari[2], M Arumugam[1*]
[1] PG and Research Department of Botany, J.J College of Arts and Science (Autonomous), Pudukkottai, Tamil Nadu, India
[2] PG and Research Department of Physics, Government Arts College for Women, Pudukkottai, Affiliated to Bharathidasan University, Thiruchirappalli, Tamil Nadu, India
*Corresponding Author: M Arumugam

Abstract
Curcuma pseudomontana is one of the plant species reported from the Western Ghats of India, belonging to the family *Zingiberaceae*, with ethno botanical values. In the present investigation, methanol extract of *C.pseudomontana* rhizome *was* analyzed by preliminary phytochemical and gas chromatography-mass spectrometry (GC–MS) to identify the important phytochemical constituents. The preliminary phytochemical analysis revealed the presence of tannins, flavonoids, alkaloids, glycosides, reducing sugars, and sterol from rhizome extracts. The GC–MS analysis of ethyl acetate and methanol extracts from methanol extract from rhizomes of *C.pseudomontana* detected the presence of 104 phytochemical compounds. The results of the present study will create a way for the invention of herbal medicines for several ailments by using *C.pseudomontana* plants, which may lead to the development of novel drugs.

Keywords: *Curcuma pseudomontana*, rhizome extracts, GC–MS, phyto-chemical

Introduction
India is one of the mega-diversity centers harboring a multitude of medicinal plant species each presumably studied with yet unknown genetic and chemical variations of economic importance. Out of an estimated 17,000 higher plant species present in India, more than 1000 species are used over several centuries in the traditional systems of medicine *viz*. Ayurveda, Siddha, and Unani. The native people and tribal folks spread across the world make use of more than 7000 plant species for their medicinal purposes (Pushpangadan *et al*., 1997) [18]. Nearly 75% of the herbal drugs that have been used in the world are available in their natural state in India (Jain, 1979) [11]. Plants are the traditional medicinal sources for many chemicals used as pharmaceuticals, bio-chemicals, fragrances, food colors, and flavors (Leung, 1980) [14]. Medicinal plants are the best area of interest to the researcher in the field of phytochemistry, pharmacology and biotechnology, etc., Most of the drug industries depend in part of plants for the production of active pharmaceutical compounds. In modern health care systems, secondary metabolites of plants constitute an ever more important source of modern pharmaceutical drugs, and they are becoming an increasingly valuable commodity in expanding market for herbal remedies. Investigations into the chemical and biological activities of the plant during the past two centuries have yielded numerous compounds for the development of modern synthetic phytochemistry and the emergence of medicinal chemistry as a major route for the discovery of novel and more effective therapeutic agents (Alan Sheeja *et al*., 2012; Shabir *et al*., 2013**)** [3, 19]. The identification of active compounds is an essential requirement for quality control and dose determination of plant-based drugs. Research in the pharmacognosy of medicinal plants has also involved assays of bio-activity, determination of the potential mode of action, and target site for active compounds (Briskin, 2000; Ahmad *et al*., 2011; Charles *et al*., 2013) [7].

The *Curcuma* is a genus of economically and medically important. Despite its economic importance, the genus is poorly understood, botanically and chemically. In addition to *C.longa*, the genus includes other economically important species such as *C.aromatica*, C.*pseudomontana*, *C. ochrorhiza*, *C. pierreana*, *C. zedoaria*, *and C. caesia*, etc., used in folk medicines of the Southeast Asian nations. The Latin name *Curcuma* is derived from the Arabic word kurkum, which meant Saffron in olden times. Saffron and Turmeric were the most popular yellow dye-yielding plants in the ancient world, commercially important for dying clothes, fibers, food. Turmeric is popularly known as Indian Saffron in many vernacular languages.

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This species is a rhizomatous herbaceous perennial, which is found in usually moist shady places on the fringes of wet forests or grasslands, in riparian areas, at moderately high latitude along the western side of the Western Ghats (Manguly and Sabu, 1987) [16]. The taxon occurs both in moist deciduous forests and semi-evergreen forests (Molur *et al*., 1997) [17]. This plant should be included in belonging to the family-Zingiberaceae, order-Zingiberales. This plant is commonly called Hill Turmeric. In Tamil it is called Kattu Manjal, Hindi-Kachura, Malayalam-Kattu Manjal, Telugu-Adavi Pasupu. The present study is aimed to analyze the phytochemical constituents of the rhizome and find to analyze the bioactive compounds using GC-MS of the rhizome of *Curcuma pseudomontana.*

Materials and Methods
The phytochemical screening of *C. pseudomontana* was performed; the dried powdered rhizome was extracted using

the Soxhlet apparatus using methanol. The methanol extract was filtered and concentrated using a rotary vacuum evaporator and the dried extract was stored in an airtight container.

Test for terpenoids (Salkowski test)
To 0.5g of each of the extracts was added 2ml of chloroform, Concentrated Sulphuric acid (3 ml) was carefully added to form a layer. The reddish-brown coloration of the interface indicates the presence of terpenoids (Ayoola *et al.*, 2008) [5].

Test for flavonoids
4ml of extract solution was treated with 1.5ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of Concentrated Hydrochloric acid were added and red color was observed for flavonoids and orange color for flavones (Harborne, 1973; Sofowora, 1993) [10, 20].

Test for alkaloids
Wagner's test: Potassium iodide (2 g) and iodine (1.27 g) were dissolved in distilled water (5 mL) and the solution was diluted to 100 ml with distilled water. Few drops of this solution were added to the filtrate; a brown-colored precipitate indicates the presence of alkaloids. (Joshi *et al*., 2013, Abdullahi *et al*., 2013) [12, 1].

Test for tannins
About 0.5g of the extract was boiled in 10ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride were added and observed for brownish green or a blue-black coloration (Antherden, 1969) [4].

Test for saponins
To 0.5g of extract was added 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion (Just *et al*., 1998) [13].

Test for glycosides (Keller Killiani's test)
Another portion of the extract was hydrolyzed with hydrochloric acid for a few hours in a water bath and the hydrolysate was subjected to Legal's to detect the presence of different glycosides. To the hydrolysate, 1 ml pyridine and few drops of sodium nitroprusside solution were added, and then it was made alkaline with sodium hydroxide solution.
The formation of purple color indicated the presence of glycosides.

Test for Reducing Sugar
F*ehling test:* Mixed 1 ml of Fehling's A and Fehling's B solutions, boiled for one minute. Added equal volume of test solution. Heated in a boiling water bath for 5–10 min. First a yellow then a brick red ppt. was observed (Boxi *et al*., 2010) [6].

Test for Steroid
Added 2 ml of acetic anhydride and 2 ml of Concentrated H_2SO_4 to 5 ml of the extract. The change of color from violet to blue confirms the presence of steroids (Boxi *et al*., 2010) [6].

Test for anthrquinone
To the extract solution (1 mL), 5% H_2SO_4 (1 mL) was added. The mixture was boiled in a water bath and then filtered. The filtrate was then shaken with an equal volume of chloroform and kept to stand for 5 min. Then a lower layer of chloroform was shaken with half of its volume with dilute ammonia. The formation of the rose-pink to red color of the ammoniacal layer indicates anthraquinone glycosides (Joshi *et al*., 2013) [12].

Gas chromatography-Mass spectrometry (GC-MS)
GC-MS of the extract was performed on HP 6890/5973-GC-MSD-D5 at 75eV and 250ºC. The GC column condition used was: HP-5 (DB5), fused silica capillary – 0.32mm x 30m with film thickness 0.25; carrier gas –helium, length of the column – 30m, flow rate – 1.4ml/min. Temperature program: initial temperature 60°C for 1 min. and then heated at the rate of 3°C/min to 246°C. Run time is 56 min. The components were analyzed and structures of various components were ascertained with the help of Wiley Library 275 combined with the analyzer.

Results and Discussion
The availability of preliminary phytochemicals in *C.pseudomontana* rhizome was screened and tabulated in Table.1. Thus, the rhizome of the species showed the presence of tannins, flavonoids, alkaloids, glycosides, reducing sugars, and sterol. But terpenoids and anthraquinones were not present. The compounds present *in C.pseudomontana* were flavonoids, glycosides, and tannins, thus from the data, it is evident that the study plant could make useful in treating different ailments and having potential for providing the useful drug to have curative activity against deceases producing pathogens. Preliminary research indicates that flavonoids may modify allergens, viruses, carcinogens and so maybe biological "response modifiers". *In vitro* studies of flavonoids have displayed anti-allergic, anti-inflammatory, antimicrobial (Cushnie and Lamb, 2005) [9]. Hence, most of the secondary metabolites have been shown to present interesting biological and pharmacological activities and are used as drug agents to treat a variety of diseases or serve as the initial opening in the improvement of modern medium **(**Verpoorte, 1998) [21]. Therefore, it can be used pharmacologically to develop new compounds for health benefits (Sofowara, 1993).

Table 1: Preliminary Phytochemical analysis of *C.pseudomontana* rhizome

S.no	Name of the compound	Test	Availability
1.	Terpenoids	Salkowski test	_
2.	Flavonoids	Shinoda Test	++
3.	Alkaloids	Wagner's test	++
4.	Tannin	Stiasny methods	+++
5.	Saponins	Foam Index: positive if >100	++
6.	Glycosides	Keller-Kiliani test	+++
7.	Reducing sugars	Fehling's test	++
8.	Steroid	Liebermann–Burchard	++
9.	Anthraquinone	Borntrager's	-

(High Concentration: +++; Moderate Concentration: ++;Low Concentration; Absent:-)

Gc-Ms study

The pale yellow-colored essential oil was obtained by hydro distillation of the mature rhizomes. The details of the major chemical components of the essential oil are given in table.10. The GC–MS analytical study of the essential oil has resulted in the identification of 104 components representing 99.43% of the total oil. The major components of the essential oil (EO) were benzene (1,5 dimethyl-4-hexenyl)-4-methyl benzene (7.27%), b-elemenone (22.14%), germacrone (15.15%), pseudocumenol (20.65%) and 2-(4-methoxy phenyl)N,N trimethyl-1-pyrrolamine (13.12%), Benzene1-(1,5 dimethyl-4-hexenyl)-4-methyl (7.27%), Benzofuran (2.89), Allospathulenol (2.69), 5-Ethyl-1,3-dimethylindan (1.98%), Germacrene (1.83%), Isopropyl-1,2,4-trimethylbenzene (1.55%) etc., Gas Chromatography-Mass Spectroscopy (GC-MS) plays a key role in the analysis of unknown components of plant origin. Generally, the plant materials are highly complex, which makes GC-MS well suited for their analysis because of its high sensitivity and selectivity. GC-MS ionizes compounds and measures their mass numbers. It provides additional information on the structure of these profiles. The overall evaluation of the compounds present in the plant extract was analyzed by using GC-MS. Alcohol was used as a solvent for the separation of bioactive compounds present in the plant leaves. Totally 104 compounds were identified in *C.pseudomontana* rhizome. The major components of the essential oil (EO) were benzene (1,5 dimethyl-4-hexenyl)-4-methyl- benzyne (7.27%), b-elemenone (22.14%), germacrone (15.15%), pseudocumenol (20.65%) and 2-(4-methoxy phenyl)N,N trimethyl-1-pyrrolamine (13.12%), Benzene1-(1,5 dimethyl-4-hexenyl)-4-methyl (7.27%), Benzofuran (2.89), Allospathulenol (2.69), 5-Ethyl-1,3-dimethylindan (1.98%), Germacrene (1.83%) have the property of anti-inflammatory activity and reducing blood cholesterol (Roussis-Vassilios *et al.*, 2000), Isopropyl-1,2,4-trimethylbenzene (1.55%) etc., Among the identified phytochemicals, Germacrene have the property of anti-inflammatory activity and reducing blood cholesterol (Roussis-Vassilios *et al*, 2000). β-Elemenone and Phenol,2-methyl-5-(1,2,2-trimethylcyclopentyl) have the property of antimicrobial activity and antioxidant activity (Tung *et al.*, 2008; Shanthi and Nelson, 2013). The phytol was reported in *Mentha spicata* for its antimicrobial and antiviral activities, strong antioxidant, and antitumor action (Mckay and Blumberg, 2006). Germacrone enhanced the activation of the JAK2/STAT3 signaling pathway and attenuated the germacrone-induced apoptosis. Germacrone resulted in apoptosis and an increase in ROS in response to germacrone exposure also; it induces apoptosis through the JAK2/STAT3 signaling pathway (Amal Banafa *et al.*, 2013).

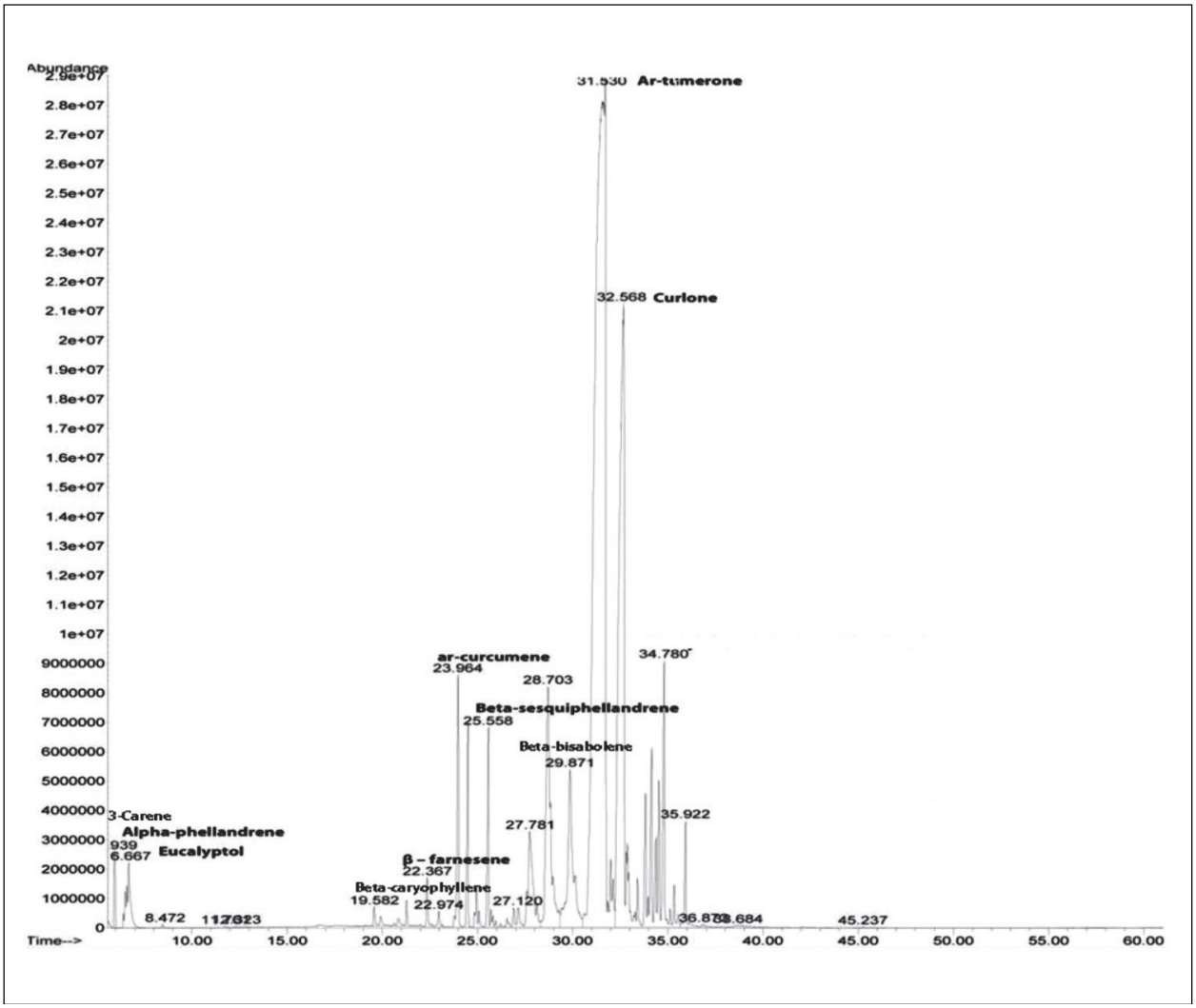

Fig 1: Phytoconstituents detected in the methanol leaf extract of *C.pseudomontana* using gas chromatography-mass spectrometry.

Table 2: Chemical compositions of the rhizome extract of *Curcuma pseudomontana.*

S. no.	Constituents of phytochemical	Area peak (%) of EO
1	Benzene1-(1,5 dimethyl-4-hexenyl)-4-methyl-	7.27
2	Benzofuran	2.89
3	Gamma-Curcumene	0.72
4	Germacrene	1.83
5	3-Ethyl-6-(methoxycarbonyl)-naphthol	0.53
6	3,7-Cyclodecadien-1-one	0.45
7	β-Elemenone	22.14
8	Germacrone	15.15
9	Phenol,2-methyl-5-(1,2,2-trimethylcyclopentyl)-	0.21
10	Isopropyl-1,2,4-trimethylbenzene	1.55
11	2,2'-Butenyl-mesitylene	0.35
12	Isospathulenol	0.62
13	Trans-β-Farnesene	0.52
14	Camphor	0.34
15	Trans-Caryophllene	0.17
16	Bicyclo[2.2.1]heptan-2-one	0.66
17	Trans-β-Elemene	0.95
18	Pseudocumenol	20.65
19	2-(4-Methoxy phenyl)N,N trimethyl-1-pyrrolamine	13.12
20	Allospathulenol	2.69
21	5-Ethyl-1,3-dimethylindan	1.98
22	1,6-Heptadiene	1.39

Conclusion

As an economically important medicinal plant, it is used in several ayurvedic preparations. Preliminary phytochemical analysis revealed that various solvent extracts of *C.pseudomontana* showed the presence of terpenoids, flavonoids, tannins, saponins, and phenolic compounds (Curcuminoid). *Curcuma pseudomontana* yielded 104 compounds in GC-MS analysis. Two compounds namely n-Hexadecanoic acid and 2-Propenoic acid, 3-(4-methoxyphenyl), ethyl ester were common for all extracts. Some compounds are known for activities like anticancer, antitumor, antioxidant, hypocholesterolemic, anti-inflammatory, Antiochene, antiseptic, antiandrogenic, anti-microbial, sedative, anesthetic, and analgesic, etc. Some other compounds have herbicidal, nematicidal, fungicidal, insecticidal, pesticidal, and termiticidal properties.

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