Pharmacogn J. 2017; 9(6):952-956
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com | www.journalonweb.com/pj | www.phcog.net

Original Article

Phytochemical Screening, GC-MS, FT-IR Analysis of Methanolic Extract of *Curcuma caesia* Roxb (Black Turmeric)

Muthukumaran Pakkirisamy, Suresh Kumar Kalakandan* and Karthikeyen Ravichandran

ABSTRACT

Aims: The purpose of the current study is to monitor the phytochemical constituents in the *Curcuma caesia* Roxb.by GC MS and FT-IR analysis. **Methods:** The Rhizomes of *Curcuma caesia* Roxb was extracted with Methanol at room temperature for 8 h. The bioactive compounds of *Curcuma caesia* Roxb have been evaluated using GC-MS and FT-IR. **Results:** Preliminary phytochemical analysis revealed the presence of tannins, terpenoids, flavonoid, alkaloid, phenol, phytosterol Quinones and saponins. Totally 15 compounds were identified and the chromatograph showed peaks with individual compounds. The major constituents were identified in the Methanolic extract were α-Santalol (46.90%), Retinal (10.72%), Ar-tumerone(10.38%), Alloaromadendrene (5.93%), Megastigma-3,7(E),9-triene (4.80%), Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl(4.38%) , 5,8,11,14,17-Eicosapentaenoic acid, methyl ester, (all-Z)-(4.26%) Tricyclo[8.6.0.0(2,9)]hexadeca-3,15-diene, trans-2,9-anti-9,10-trans-1,10 (3.26%) and many other compounds were identified as low level. The FTIR analysis confirmed the presence of N-H , O-H , C=C , C-H, C-O and CH_3 functional groups. **Conclusion:** The result of this study offer a platform of using *Curcuma caesia* Roxb as herbal alternative for various diseases and it can be used as functional and pharmaceutical food.

Key words: Phyto chemical, *Curcuma caesia* Roxb, GC MS, FT-IR, α-Santalol and Retinal.

Muthukumaran Pakkirisamy, Suresh Kumar Kalakandan* and Karthikeyen Ravichandran

Department of Food Safety and Quality Testing Laboratory, Indian Institute of Food Processing Technology, Thanjavur, Tamil Nadu, INDIA.

Correspondence

Suresh Kumar Kalakandan

Head i/c, Department of Food Safety and Quality Testing Laboratory, Indian Institute of Food Processing Technology, Thanjavur, Tamil Nadu, INDIA.

Phone: +91-9655397245

E-mail: sureshkumar.k@iifpt.edu.in, kumaran.bio14@gmail.com

History

- Submission Date: 27-07-2017;
- Review completed: 11-08-2017;
- Accepted Date: 31-08-2017.

DOI : 10.5530/pj.2017.6.149

Article Available online
http://www.phcogj.com/v9/i6

INTRODUCTION

Curcuma Linn. is a large genus belonging to the family *Zingiberaceae*. It comprises about 70 species of rhizomatous herbs distributed mostly in Southeast Asia as wild and cultivated plants. *Curcuma caesia* is a perennial herb with bluish-black rhizomenative to Northeast and Central India. *Curcuma caesia* (Black Turmeric) is also sparsely originated in Papi Hills of East Godavari, West Godavari, and Khammam Districts of Andhra Pradesh. The rhizomes of *Curcuma caesia* have a high economic importance because of its putative medicinal properties. The rhizomes are used in the treatment of smooth muscle relaxant activity[1] haemorrhoids, leprosy, asthma, cancer, epilepsy, fever, wound, vomiting, menstrual disorder, anthelmentic, aphrodisiac, inflammation, gonorrhoeal discharges, etc.[2]

The rhizomes of the plant are aromatic in nature. The inner part of the rhizome is bluish-black in colour and emits a characteristic sweet smell, due to presence of essential oil.[3] Traditionally, the rhizomes of *Curcuma caesia* Roxb. Are used in treating leucoderma, tumors , asthma, piles, bronchitis etc. The paste is applied on bruises, contusions and rheumatic pains.[4] Fresh rhizome decoction is used as antdiarrhoeiaric and to get relief from stomach ache. The fresh rhizome paste of *curcuma caesia* is Roxb applied during the snake bite and scorpion bite.[5,6]

The advances in analytical techniques, including GC-MS and FT-IR that were powerful tools for identification and determination of phytochemicals compounds. The present study was carried out the bioactive compounds present in the *Curcuma caesia* Roxb in methanol extract with the aid of GC-MS and FT-IR techniques, which may provide an insight in its use of traditional medicine.

MATERIAL AND METHODS

Plant material collection and extraction

Rhizomes of *Curcuma caesia* Roxb. were collected from Thanjavur, Tamil Nadu, and India. The collected Rhizome was shade dried, powered and extracted with methanol using Soxhlet apparatus for 8 hours. The extracts were filtered and filtrates were concentrated under reduced pressure at 40° C using a rotary flash evaporator and stored at 4°C until use for phytochemical screening.

Phytochemical Screening

Phytochemical analysis was carried out for identification of tannins, terpenoids, flavonoid, alkaloid, phenol, phytosterol, Quinones and saponins according to standard methods.[7,8]

Cite this article: Pakkirisamy M, Kalakandan SK and Ravichandran K. Phytochemical Screening, GC-MS, FT-IR Analysis of Methanolic Extract of *Curcuma caesia* Roxb (Black Turmeric). Pharmacog J. 2017;9(6):952-6.

GC –MS Analysis

Preparation of extract

Rhizome powder of *Curcuma caesia* Roxb were shade dried. 20 g of the powdered tubers were soaked in 95% ethanol for 12 h. The extract was then filtered through Whatmann filter paper No.41 along with 2 gm sodium sulfate to remove the sediments and traces of water in the filtrate. Before filtering, the filter paper along with sodium sulphate was wetted with 95% ethanol. The filtrate was then concentrated by bubbling nitrogen gas into the solution. The extract contained both polar and non-polar phytocomponents of the plant material used.

GC Condition and Identification of Compounds

The sample was investigated through Gas Chromatography Mass Spectrometry/Mass Spectrometry Electron Ionization (GC-MS/EI) mode. The GC-MS/MS is a Scion 436- GC Bruker model coupled with a Triple quadruple mass spectrophotometer with fused silica capillary column BR-5MS (5% Diphenyl/95% Dimethyl polysiloxane) and Length: 30m; Internal diameter: 0.25 mm; Thickness: 0.25 µm. Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1 ml/min and an injection volume of 2 µl was employed (split ratio of 10:1). The injector temperature 250°C; ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C/min, to 200°C, then 5°C/min to 280°C, ending with a 9 min isothermal at 280°C and total GC running time was 41 min.[9] This last increase was to clean the column from any residues. The mass spectrometer was operated in the positive electron ionization (EI) mode with ionization energy of 70eV. The solvent delay was 0-3.0 min. A scan interval of 0.5 seconds and fragments from m/z 50 to 500 Da was programmed. The inlet temperature was set at 280 °C, source temperature 250 °C. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was MS Work station 8. The NIST Version 2.0 library database of National Institute Standard and Technology (NIST) having more than 62,000 patterns was used for identifying the chemical components. The GC-MS/MS was performed by Food Safety and Quality Testing Laboratory, Institute of crop processing technology, Thanjavur.

FTIR Spectroscopic Analysis

Fourier transform infrared spectrophotometer (FTIR) is perhaps the most powerful tools for identifying the types of chemical bonds (functional groups) present in compounds. Dried powders of different solvent extracts of each plant material were used for FTIR analysis. 10mg of the dried extract powder was encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample disc. The powdered sample of each plant specimen was loaded in FTIR Spectroscope (Shimadzu, IR Affinity1, Japan), with a scan range from 400 to 4000 cm-1 with a resolution of 4cm-1.

RESULTS AND DISCUSSION

Plants are very important source of potentially useful bioactive principles for the development of new chemotherapeutic agents.[10] The biological and pharmacological properties of many plants are still unknown. World over, the scientists are exploring the potential of utilizing pharmacologically active compounds from medicinal plants.[11] Herbal medicines are used by 80% of the people worldwide due to its high efficiency, cheap cost, nonnarcotic nature and fewer side effects.[12]

In the present study, the exploration of phytochemical screening with Methanol extract of *Curcuma caesia* revealed the presence of carbohydrate, flavonoid, steroid, phenol, alkaloid, tannin, amino acid, terpenoid and glycoside compounds which are known to have remedial activity against diseases producing pathogen. Therefore it can be used pharmacologically to develop new compounds for health benefit (Table 1).

Table 1: Phytochemical constituents present in Methanolic extracts of *Curcuma caesia* Roxb

S.No	Phytochemicals	*Curcuma caesia* Methanol Extract
1	Tannins	+
2	Terpenoids	+
3	Flavonoid	+
4	Phenol	+
5	Phytosterol	+
6	Saponins	+

Table 2: Phytocomponents identified in the Methanolic extract of the *Curcuma caesia* Roxb By GC-MS

S.No	RT	Name of the compound	Molecular formula	MW	Peak area (%)
1	4.14	(+)-2-Bornanone	$C_{10}H_{16}O$	152	0.91
2	4.39	Isoborneol	$C_{10}H_{18}O$	154	0.40
3	7.85	Alloaromadendrene	$C_{15}H_{24}$	204	0.63
4	9.13	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	$C_{15}H_{22}$	202	4.38
5	9.33	Tricyclo[8.6.0.0(2,9)]hexadeca-3,15-diene, trans-2,9-anti-9,10-trans-1,10-	$C_{16}H_{24}$	216	3.26
6	9.71	trans-Sesquisabinene hydrate	$C_{15}H_{26}O$	222	1.39
7	10.74	α-Santalol	$C_{15}H_{24}O$	220	46.90
8	11.46	Ar-tumerone	$C_{15}H_{20}O$	216	10.38
9	11.93	Megastigma-3,7(E),9-triene	$C_{13}H_{20}$	176	4.80
10	13.18	5,8,11,14,17-Eicosapentaenoic acid, methyl ester, (all-Z)-	$C_{21}H_{32}O_2$	316	4.26
11	15.57	Retinal, 9-cis-	$C_{20}H_{28}O$	284	10.72
12	16.29	Androstenediol	$C_{19}H_{30}O_2$	290	2.85
13	17.15	(+)-2-Bornanone	$C_{24}H_{40}O_4$	392	1.03
14	17.32	Isoborneol	$C_{24}H_{32}O_4$	384	2.17
15	20.66	Alloaromadendrene	$C_{23}H_{32}O_6$	404	5.93

Phytochemical constitutes of plants serves as defense mechanism against by many microorganisms. The therapeutic properties of medicinal plants are possibly due to the presence of various secondary metabolites.[13] Thus the preliminary screening test may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and improvement.

The compounds present in the methanolic extract of *Curcuma caesia*, were identified by GC-MS analysis (Figure 1). The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) are presented in Table 2. Seventeen compounds were identified in methanolic extract by GC-MS. The major components present in the *Curcuma caesia* (Black turmeric) were α-Santalol (46.90%), Retinal (10.72%), Ar-tumerone(10.38%), Alloaromadendrene (5.93%), Megastigma-3,7(E),9-triene (4.80%), Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl(4.38%), 5,8,11,14,17-Eicosapentaenoic acid, methyl ester, (all-Z)-(4.26%) Tricyclo [8.6.0.0(2,9)]hexadeca-3,15-diene, trans-2,9-anti-9,10-trans-1,10(3.26%) and various other compounds were identified as low level . These phytochemicals are responsible for various pharmacological actions like antimicrobial and anti-oxidant anti-inflammation, Anti-cancer, Hepato protective, Diuretic, Antiasthma activities etc (Table 3). *Curcuma caesia* has medicinal value the presence of these major constituents.[14]

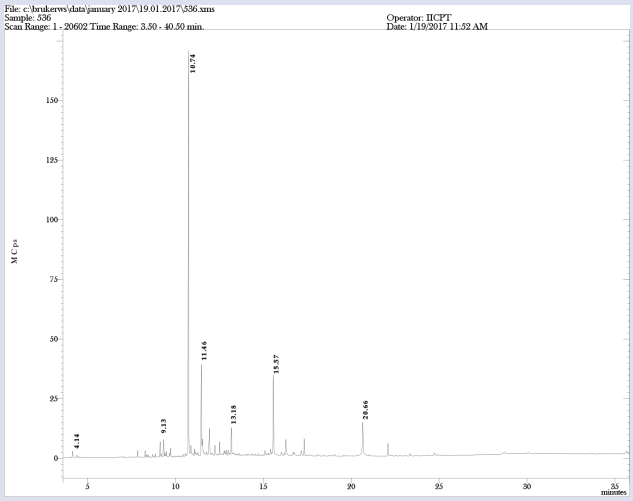


Figure 1: GC-MS analysis of *Curcuma caesia* Roxb of methanol extract.

Table 3: Activity of phyto-components identified in *Curcuma caesia* Roxb by GC-MS.

S.NO	Name of the Compound	Compound nature	Activity
1	(+)-2-Bornanone	Monoterpene oxide	Anti-tumor, Analgesic Antibacterial, Anti-inflammatory Sedative, Fungicide
2	Isoborneol	Monoterpene alcohol	Anti-tumor, Analgesic Antibacterial, Anti-inflammatory Fungicide, Sedative, Antipyretic, Antifeedent, Candidicide, Hepatoprotective, Pesticide, Perfumery Tranquilizer, Myorelaxant, Antibronchitic
3	Alloaromadendrene	Sesquiterpene	Anti-tumor, Analgesic Antibacterial, Anti-inflammatory Sedative, Fungicide
4	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	Color pigments	Skin care products Anti-inflammatory Anticancer, Antioxidant
5	Tricyclo[8.6.0.0(2,9)]hexadeca-3,15-diene, trans-2,9-anti-9,10-trans-1,10-	Steroid	Antimicrobial, Anti-inflammatory Anticancer, Diuretic, Antiasthma Haepatoprotective
6	trans-Sesquisabinene hydrate	Sesquiterpene alcohol	Anti-tumor, Analgesic Antibacterial, Anti-inflammatory Fungicide, Sedative, Antipyretic, Antifeedent, Candidicide, Hepatoprotective, Pesticide, Perfumery, Tranquilizer, Myorelaxant, Antibronchitic
7	α-Santalol	Sesquiterpene oxide	Anti-tumor, Analgesic Antibacterial, Anti-inflammatory Sedative, Fungicide
8	Ar-tumerone	Ketone compound	Anti-tumor, Analgesic Antibacterial, Anti-inflammatory Sedative, Fungicide, Antiarthritic Antidote for arsenic, Anti HIV Anticancer,
9	Megastigma-3,7(E),9-triene	Alkene compound	Anticancer, Antitumor, Expectorant, Memory enhancer
10	5,8,11,14,17-Eicosapentaenoic acid, methyl ester, (all-Z)-	Unsaturated fatty acid ester	Cardio protective
11	Retinal, 9-cis-	Vitamin A precursor	Anticancer, Eye protective
12	Androstenediol	Steroid	Antimicrobial, Anti-inflammatory, Anticancer, Diuretic, Antiasthma Haepatoprotective
13	(+)-2-Bornanone	Acidic compound	Reduce gallstone formation, Chemo preventive
14	Isoborneol	Steroid	Cytotoxic steroid, Anti-inflammatory
15	Alloaromadendrene	cardiac glycoside	Inhibit membrane protein, Strengthen the failing heart

Table 4: *FTIR* Peak Values of Methanolic Extract *of Curcuma caesia* Roxb.

S. NO	PEAK VALUES	FUCTIONAL GROUPS
1	3287.28	N-H
2	2923.88	O-H
3	1635.03	C=C
4	1324.97	C-H
5	1149.68	C-O
6	1077.21	C-O
7	1016.05	CH_3

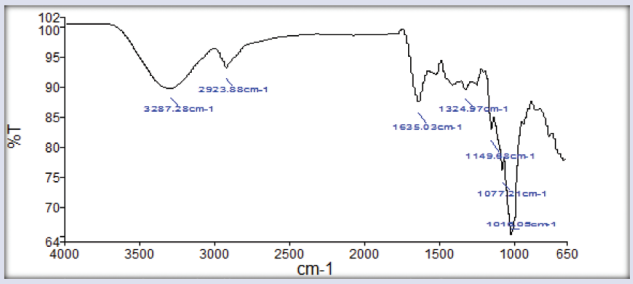


Figure 2: *FTIR* analysis of *Curcuma caesia* Roxb.

The FT-IR spectrum was used to identify the functional groups of the active components present in extract based on the peaks values in the region of IR radiation. When the extract was passed into the FT-IR, the functional groups of the components were separated based on its peaks ratio. The results of FT-IR analysis confirmed the presence of N-H, O-H, C=C, C-H, C-O and CH_3 functional groups (Figure 2 and Table 4). FTIR spectroscopy is proved to be a reliable and sensitive method for detection of bio molecular composition.

CONCLUSION

The present work has been performed to establish the various Phyto-chemical, GCMS and FTIR parameters, which could serve as important and has commercial interest in both research institutes and pharmaceuticals companies for the manufacturing of the innovative drugs. This primary information will facilitate in conducting further studies on discovery of bioactive constituents, resolve of their efficacy by *in vivo* studies and demonstration of their safety and efficacy in clinical trials.

ACKNOWLEDGEMENT

The authors are thankful to **Dr. C. Anandharamakrishnan,** Director, Indian Institute of Food Processing Technology, Thanjavur, Tamil Nadu, India for providing all the facilities to conduct this research work and also thankful to the members of Department of Food Safety and Quality Testing division of for their support.

CONFLICT OF INTEREST

There are no conflicts of interest.

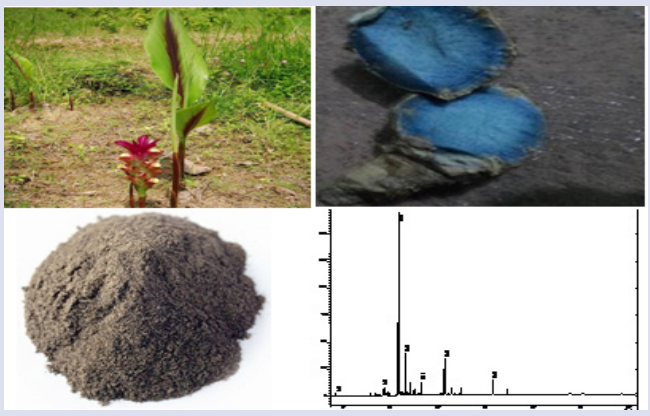
ABBREVIATION USED

GC MS: Gas chromatography–mass Spectrometry; **FT-IR:** Fourier transform- infrared.

REFERENCES

1. Arulmozhi DK, Sridhar N, Veer-Anjaneyulu A, Arora SK. Preliminary mechanistic studies on the smooth muscle relaxant effect of hydro alcoholic extract of Curcuma *caesia*. J Herb Pharmacotherapy. 2006;6(3-4):117-24.
2. Sasikumar B. Genetic Resource of Curcuma: Diversity, Characterization and Utilization. Plant Genet Resource. 2005;3(2):230-51.
3. Pandey AK, Chowdhary AR. Volatile constituents of rhizome oil of *Curcuma Caesia*. Flavour Fragr J. 2003;18(5):463-5.
4. Sarangthem K, Haokip MJ. Bioactive components in Curcuma *caesia* Roxb. grown in Manipur. The Bioscan. 2010;5:113-5.
5. Kagyung, Gajurel PR, Rethy P, Singh B. Ethnomedicinal plant used for gastrointestinal diseases by Adi tribe of Dihang-Dibang biosphere reserve in Arunachal Pradesh. J. Trad. Know. 2010;9:496-501.
6. Tag H, Das AK, Loyi H. Anti-inflammatory plant used by Khanti tribes of Lohit district in Arunachal Pradesh. Natural Product Radiate. 2007;4:340-3.
7. Evans WC. Trease and Evans pharmacognosy15th eds. Elsevier India Private Limited. Noida. 2008: 3-4.
8. Harborne JB. Phytochemistry. 4th eds. Academic Press. London. 1993:89-131.
9. Kumaravel S, Muthukumaran P, Shanmugapriya K. Chemical composition of *Trigonella foenumgraecum* through gas chromatography mass spectrometry analysis. Journal of Medicinal Plants Studies. 2017;5(3):1-3.
10. Tona L, Kambu K, Ngimbi N, Cimanga K, Vlietinck AJ. Antiamoebic and phytochemical screening of some Congolese medicinal plants. J Ethnopharmacology. 1998;61(1):57-65.
11. Karmegam N, Mani J, Subbiah K. Synergistic antibacterial activity of four medicinal plants collected from Dharapuram taluk of Tirupur district, south India. J Plant Sci. 2012;24:328.
12. Ahmad I, Beg AZ. Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. J. Ethnopharmacology. 2001;74(2):113-23.
13. Paliwal P, Pancholi SS, Patel RK. Pharmacognostic parameters for evaluation of the rhizomes of Curcuma *caesia*. J Adv Pharm Tech Res. 2011;2(1):56-61.
14. Sonjit D, Prodyut M, Kamaruz Z. *Curcuma Caesia* Roxb. and It's Medicinal Uses: A Review. International Journal of Research in Pharmacy and Chemistry. 2013;3:370-5.

GRAPHICAL ABSTRACT



SUMMARY

- Herbal medicines are used by 80% of the people worldwide due to its high efficiency, cheap cost, nonnarcotic nature and fewer side effects
- The therapeutic properties of medicinal plants are possibly due to the presence of various secondary metabolites
- The present work has been performed to establish the various Phyto¬chemical, GCMS and FTIR parameters, which could serve as important and has commercial interest in both research institutes and pharmaceu¬ticals companies for the manufacturing of the innovative drugs.

ABOUT AUTHORS

Dr. K.Suresh Kumar, Associate Professor and Head i/c of Department of Food Safety and Quality Testing, Indian Institute of Food Processing Technology, Thanjavur, Tamil Nadu, India.

Dr.P.Muthukumaran, Senior Research Fellow, Department of Food Safety and Quality Testing, Indian Institute of Food Processing Technology, Thanjavur, Tamil Nadu, India.

Dr.R.Karthikeyan, Technical Assistant, Department of Food Safety and Quality Testing, Indian Institute of Food Processing Technology, Thanjavur, Tamil Nadu, India.

Cite this article: Pakkirisamy M, Kalakandan SK and Ravichandran K. Phytochemical Screening, GC-MS, FT-IR Analysis of Methanolic Extract of *Curcuma caesia* Roxb (Black Turmeric). Pharmacog J. 2017;9(6):952-6.