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Phytochemical profiling of petroleum ether and chloroform extracts of *Curcuma caesia* rhizome by GC-MS and comparing their bioactivities

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Abstract

The objective of this study was to characterise the putative phytochemical constituents from petroleum ether (CC-P) and chloroform (CC-C) extracts of *Curcuma. caesia* rhizome by GC-MS analysis and to compare their bioactivities. GC-MS analysis facilitated the documentation of a total of 16 and 20 volatile constituents in CC-P and CC-C extracts respectively. Of these the major constituents present in CC-P were cycloisolongifolence, 8, 9-dehydro-9 formyl- (19.64%), 4-oxo-β-isodamascol (16.05%), 6,10-dimethyl-3- (1-methylethyl) -6-cyclodecene-1,4-dione (13.80%), 2H-cyclohepta[b]furan-2-one,6-[1-(acetyloxy)-3-oxobutyl]-3,3a,4,7,8,8a-hexahydro-7-methyl-3-methylene- (9.08%), tricycle [5.1.0.0(2,4)]oct-5-ene-5-propanoic acid,3,3,8.8-tetramethyl- (6.39%),2(3H)-benzofuranone,6-ethenylhexahydro-3,6-dimethyl-7-(1-methylethenyl)-, [3S-(3α,3aα,6α,7β,7aβ) (4.18%) and 7a-isopropenyl-4,5-dimethyloctahydroindene-4-carboxylic acid (4.01%). Similarly, the major constituents present in CC-C were cycloisolongifolence,8,9-dehydro-9-formyl-(15.69%), 6,10-dimethyl-3-(1-methylethyl)-6-cyclodecene-1, 4-dione (13.38%), 2H-cyclohepta[b]furan-2-one,6-[1-(acetyloxy)-3-oxobutyl]-3,3a,4,7,8,8a-hexahydro-7-methyl-3-methylene- (4.55%), tricycle [5.1.0.0(2,4)]oct-5-ene-5-propanoic acid,3,3,8.8-tetramethyl- (7.50%), 2(3H)-benzofuranone,6-ethenylhexahydro-3,6-dimethyl-7-(1-methylethenyl)-,[3S-(3α,3aα,6α,7β,7aβ) (6.15%), 7a-isopropenyl-4,5-dimethyloctahydroindene-4-carboxylic acid (6.43%) and 2-(4a,8-Dimethyl-6-oxo-1,2,3,4-4a,5,6,8a-octahydro-naphthalen-2-yl) propionaldehyde (6.57%). The extracts, CC-P and CC-C exhibited almost similar antibacterial and antifungal potentials but differed with respect to antioxidant activity. The IC_{50} values of CC-P and CC-C extracts for DPPH scavenging were 0.68±0.02 mg ml^{-1} and 0.13±0.01 mg ml^{-1} respectively. Together, above results provide an important basis for the isolation of bioactive compounds from *C. caesia* rhizome extract and their use as traditional medicine.

Keywords: phytoconstituents, GC-MS, bioactivities, antifungal, antibacterial, DPPH scavenging

Introduction

Plants of Zingiberaceae family are good source of antioxidants and antimicrobial bioactive substances (I-Nan *et al*. 2008; Kamazeri *et al*. 2012; Voravuthikunchai 2007). *Curcuma. caesia* is known as black turmeric for its unique black-bluish rhizome and is endemic to north east and central India (Bhupendra *et al*. 2016). The plant is cultivated across southeast Asian countries for its innumerable medicinal usage (Arulmozhi *et al*. 2006; Devi *et al*. 2015; Gupta *et al*. 2018; Jain & Parihar 2018; Jose & Thomas 2014; Karmakar *et al*. 2013; Sonjit *et al*. 2013). This has prompted researchers to document the phyto constituents of rhizome and rhizome oil of *C. caesia*. Occurrence of various compounds in *C. caesia* rhizome; rhizome oil and leaf essential oil were reported by different researchers (Singh *et al*. 2010; Mukunthan *et al*. 2014; Paw *et al*. 2019; Paliwal *et al*. 2011; Banarjee & Nigam 1984; Borah *et al*. 2019). Apart from oil, plant extracts are also known to be rich in volatile bioactive constituents. A wide range of extraction methods are reported to prepare plant extracts such as microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), supercritical fluid extraction (SFE), maceration and Soxhlet extraction (Azwanida, 2015). Of these, Soxhlet method can extract large amounts of phytochemicals with a much smaller quantity of solvent, thus allows tremendous economy in terms of time, energy and cost. Some of the common solvents used for preparing plant extracts include water, methanol, chloroform, petroleum ether, chloroform, ethyl acetate, benzene and hexane among others (Azwanida, 2015). The constituents of plant extracts may differ depending on the extraction solvent used. For example, plant extracts prepared using polar solvents like water, methanol, ethanol and ethyl acetate will be rich in polar compounds whereas those prepared in nonpolar solvents such as chloroform, petroleum ether, benzene and hexane contain mainly non polar compounds. Overall, non polar compounds are biologically more active as compared to polar ones due to their higher cellular uptake and permeability through cell membranes (Pancharoen *et al*. 2000). Some of the well-known bioactive non polar compounds isolated from plant extract of Zingiberaceae family include curcumin, turmerone and atlantone among others (Pancharoen *et al*. 2000). The choice of solvent is also known to dictate the toxicity of plant extracts. Among non polar solvents, chloroform and petroleum are preferred over benzene, hexane and others because of their lower toxicity to the biological systems. Thus, choice of the solvent for preparing plant extract is a necessary parameter to be considered for isolating bioactive compounds. There are limited studies on the identification of the volatile constituents of the extract prepared from *C. caesia* rhizome. Accordingly, the present study was aimed to identify the putative nonpolar volatile constituents from petroleum ether (CC-P) and chloroform (CC-C) extracts of *C. caesia* rhizome by GC-MS and to study their bioactivities.

Materials and methods

Reagents and chemicals

The organic solvents such as chloroform, petroleum ether, methanol and carbon tetrachloride of UV spectroscopy grade was procured from local suppliers. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) and soy lecithin were procured from Sigma-Aldrich (Steinheim, Germany). Buffer reagents like monosodium phosphate, disodium phosphate, potassium persulphate ($K_2S_2O_8$) and diammonium salt of 2,2′-azino-bis (3-ethylbenz-thiazoline-6-sulfonic acid) (ABTS) of analytical grade were procured from the local suppliers (Ravis & Company, Mumbai). Nutrient broth, potato dextrose agar medium, potato dextrose broth and dimethyl sulfoxide (DMSO) were obtained from HIMEDIA, India. Nitrous Oxide (N_2O) gas of highest purity was used for pursing of liposomal solution of soy lecithin. Reagent solutions were prepared using water from Milli-Q system (Elix-5, Merck-Millipore, Germany). Steady state absorption measurements were carried out in a quartz cuvette (1 cm × 1 cm) by employing JASCO V-630 spectrophotometer (Japan). Further, ^{60}Co γ-irradiation facility (dose rate = 9 Gy min^{-1})

of Bhabha Atomic Research Centre) was used to perform the lipid peroxidation assay. The absorbed dose for the source was validated by standard Fricke dosimetry solution.

Collection of plant material

The rhizomes of *C. caesia* were collected from Thoubal District, Manipur, North-East India during December 2017.

Extract preparation

The rhizomes were cleaned under tap water, sliced, air dried (room temperature for 15 days) and ground into fine powder of 30-40 mesh size by an electric grinder (USHA Optima Classic 500 W Mixer Grinder, 1800 RPM). Further, ~115 g of the rhizome powder was sequentially extracted with 300 ml each of petroleum ether and chloroform by a Soxhlet extractor at their respective boiling points till colourless solvents were obtained. The solvents from of the respective extracts were evaporated under reduced pressure using vacuum rotary evaporator to obtain viscous semi solid masses, dried at room temperature and stored in air tight containers at 4°C until used. The corresponding extracts so obtained were abbreviated as CC-P for petroleum ether extract and CC-C for chloroform extract (Fig. 1).

Phytochemical grouping

Qualitative phytochemical screening of the extracts was performed using the standard protocol reported earlier (Donipati & Sreeramulu 2015; Satyavam & Warjeet 2012). In brief, for alkaloid testing, extract was incubated with mixture of Mayer's reagent, Wagner's reagent and Dragendorff reagent and monitored for precipitation. For flavonoid testing, extract was incubated with magnesium ribbon and monitored for appearance of red or yellow coloration. For phenol detection, extract was mixed with 10% aqueous $FeCl_3$ and monitored for appearance of blue or green coloration. For tannin detection, extract was mixed with potassium dichromate and monitored for precipitation. For steroid detection, extract was mixed with chloroform-sulfuric acid mixture and monitored for bluish red to cherry coloration. For terpenoids detection, extract was mixed with chloroform-sulfuric acid mixture and monitored for reddish brown precipitate. For saponin detection, extract was mixed with distilled water and examined for frothing persistence. For cardiac glycosides, extract was incubated with glacial acetic acid, ferric chloride and concentrated H_2SO_4 and monitored for appearance of green coloration. For protein detection, extract was mixed with Millon's reagent and H_2SO_4 and monitored for red colour precipitate. For coumarin testing, extract was mixed with 10% NaOH and monitored for yellow colouration.

GC-MS study

GC-MS study was conducted on a GC instrument (Agilent Technologies 7890 A) coupled to mass spectrometer (AccuTOFGCv,JMS-T100GCV, JEOL, Japan) under the following experimental conditions. The instrument was calibrated every six months using perfluorokerosene (b.p. 430). About 1 µl of the extract solution in methanol was injected using helium as a carrier gas with a flow rate of 1 ml/min and split ratio of 1:10. The analytical column attached to the GC was HP5 column (length – 30 m, internal diameter - 0.25 mm, ethylacetate film thickness - 0.25 µM, Agilent Technologies India Pvt. Ltd., Bangalore) and the temperature of injector was adjusted at 250 °C. The GC oven temperature program started at 60 °C (hold for 1 min), ramped to 200 °C at 8 °C min^{-1} (1 min hold), then to 275 °C at 6 °C min^{-1} up (15 min hold), and finally, to 285 °C at 5 °C min^{-1} with 0 min hold. The total run time of GC analysis was 49 min. The data was acquired using electron ionisation (EI) positive mode at 70eV and the mass scan was performed from 40 to 700 Da. An individual peak separated by GC entered the MS and got ionized. The compounds were putatively identified by matching mass ion peaks corresponding to individual peak of chromatogram with mass ion peaks of known or reference spectra stored in National Institute of Standard and Technology (NIST), MS library Search 2.0-[Q] software. The

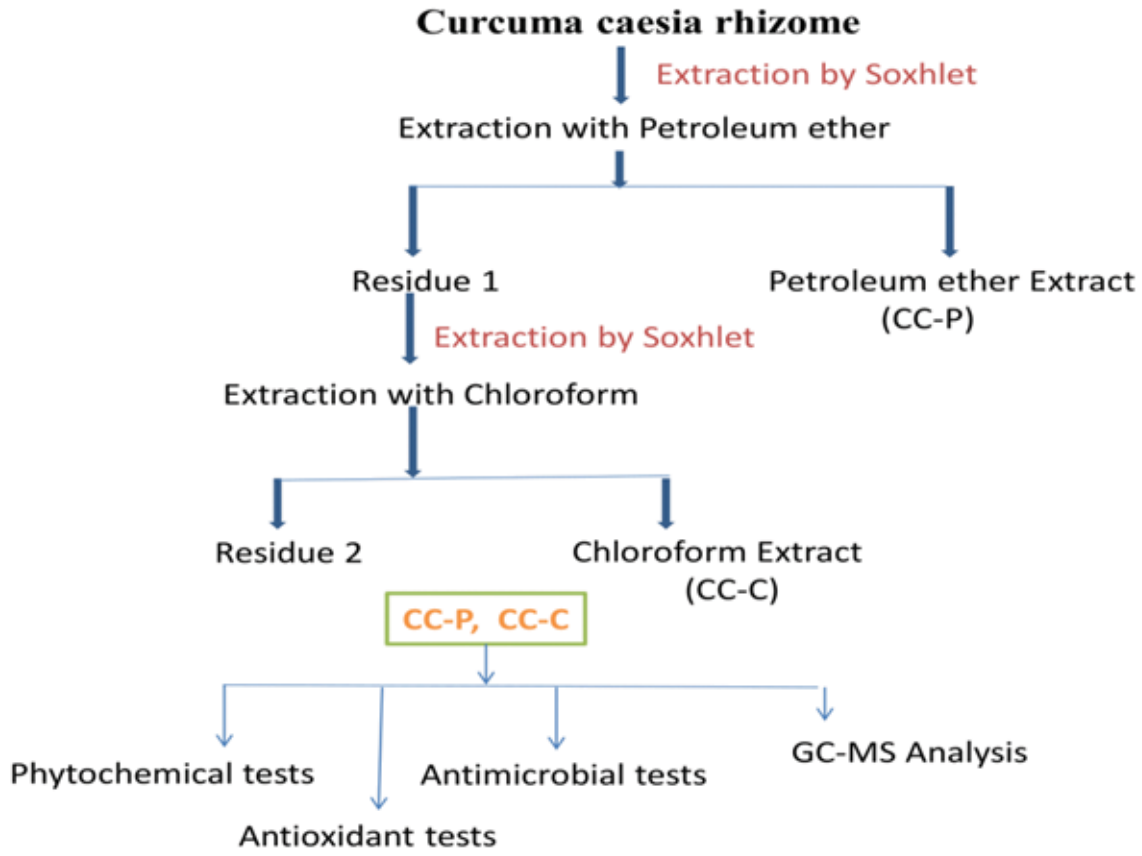

Fig. 1 Schematic diagram depicting stepwise extraction process from the rhizome of *C. caesia* using petroleum ether and chloroform as organic solvent

library search software of the GCMS system calculated three parameters, match factor (on a scale of 0-999), reverse match factor (on a scale of 0-999), and probability (% on a scale of 0-100%) to generate the hit list of putative compounds with possible matching mass spectra, chemical structure and molecular weight. From this list, the compound showing highest match factor score and probability was assigned as the chemical identity of the submitted peak of the chromatogram. The relative abundance of each chromatogram peak was calculated by normalizing its area with the total area of chromatogram.

Antimicrobial and antifungal activities

Test organisms

The antibacterial screening was performed against three gram-positive bacteria, *Bacillus subtilis* (MTCC121), *Micrococcus luteus* (MTCC 106) and *Mycobacterium smegmatis* (MTCC 6) and one gram-negative bacteria, *Escherichia coli* MTCC 739). The antifungal screening was performed against a plant pathogen *Rhizoctonia solani* (MTCC 4633) and an animal pathogen *Aspergillus niger* (MTCC 1344). The pathogenic bacterial and fungal strains were procured from IMTECH, Chandigarh, India.

Biocidal assays

The CC-C and CC-P were evaluated for antifungal and antibacterial activities by agar well diffusion method (Bauer *et al*. 1966) with some modifications. Crude extracts were solubilized in DMSO to prepare a solution with 25 µg ml^{-1} concentrations. The bacterial test pathogens and fungal test pathogens were grown in nutrient broth and potato dextrose broth respectively for 24 hours at 37 °C and

100 µl of the bacterial broths were spread onto nutrient agar media and fungal culture broths onto potato dextrose agar. Sterile cork borer was used to make wells in each plate of 6 mm diameter. About 100 µl of the samples were inoculated in 6 mm wells and the plates were incubated for 24 hours at 37 °C. Clearing zone around the well indicated antibacterial/ antifungal activity and were compared with the activity of the standards, Ampicillin (25 µg) and Fluconazole (25 µg). DMSO served as negative control.

Antioxidant activity measurements

The extracts, CC-P and CC-C were examined for antioxidant activity by measuring the scavenging of ABTS and DPPH radicals and of the inhibition of oxidative degradation of lipids or lipid peroxidation by using methods described previously (Rameshor *et al*. 2021). Due to low solubility of CC-P and CC-C extracts in aqueous system, it was first dissolved in pure methanol and subsequently the percentage of methanol in phosphate buffer (pH 7) solutions was adjusted as per the experimental conditions. In brief, DPPH (50 µM) or ABTS (120 µM) were mixed with the varying concentrations of extracts (CC-P/ CC-C) in phosphate buffer (pH 7), incubated in dark for 20 minutes (room temperature) and subjected to absorbance measurement at 518 nm and 738 nm respectively. The IC_{50} (concentration of extracts which reduces the absorbance of DPPH radicals to half of initial value) were determined from the percentage scavenging activity by using equation (1) as given below.

$$\% \text{ scavenging activity} = \frac{(A_{control} - A_{extract})}{A_{control}} \times 100 \quad (1)$$

Where, $A_{control}$ and $A_{extract}$ are the absorbance in the absence and in the presence of extracts respectively. For lipid peroxidation, liposomes (1 mg ml^{-1}) from soy lecithin were mixed with 50 µg ml^{-1} of extracts (CC-P/CC-C) in phosphate buffer (pH 7), irradiated using ^{60}Co γ-radiation (dose rate 9 Gy $minute^{-1}$) at an absorbed dose of 270 Gy and then processed for thiobarbituric acid reactive substances (TBARS) assay (Rameshor *et al*. 2021). The content of TBARS was determined by monitoring the absorbance at 532 nm and percent (%) inhibition was estimated using the following equation:

$$\% \text{ Inhibition} = \frac{([TBARS]_{control} - [TBARS]_{extract})}{[TBARS]_{control}} \times 100 \quad (4)$$

Where, $[TBARS]_{control}$ and $[TBARS]_{extract}$ are the concentrations in the absence and presence of extracts (CC-P/CC-C) respectively.

Results & discussion

Documentation of phytochemicals

The plant extracts depending on the extraction method may differ in the medicinal properties. In order to address this, CC-P (~100 mg kg^{-1}) and CC-C (~125 mg kg^{-1}) extracts were prepared from the rhizome of *C. ceasia* and the preliminary phytochemical examination of these extracts documented the existence of phytochemicals of different classes such as flavonoids, alkaloids, tannins, phenolic compounds, steroids, terpenoids, cardiacglycosides, saponins and coumarin.

Additionally, protein was also found in the CC-P extract. Based on these results, the CC-P and CC-C extracts were further subjected to the GCMS evaluation for putative identification of the volatile constituents of these extracts. The CC-P extract showed a total of 35 peaks, of which 16 matched with NIST library (Table 1). Similarly, the CC-C extract showed a total of 40 peaks, of which 20 matched with NIST library (Table 2). Together, GSMS evaluation revealed the chemical identities of a total of 16 and 20 compounds respectively from the CC-P and CC-C extracts (Tables 1 & 2**)**. Among the NIST matched compounds, 5,6-Azulenedicarboxaldehyde,1,2,3,3a,8,8a-hexahydro-2,2,8-trimethyl-,(3a*α*,8*α*,8a*α*)-(-)-; Isoaromadendrene epoxide; 4-Oxo-β-isodamascol; 4,8,13-Cyclotetradecatriene-1,3-diol,1,5,9-trimethyl-12-(1-methylethyl)- and 9,12,15-Octadecatrienoic acid-2,3-bis (acetyloxy) propyl ester, [Z,Z,Z]- were

Table 1. GC-MS identification of phytochemicals present in petroleum ether extract (CC-P) of the rhizome of *C. caesia*

Sl. No.	RT	Area [Intens. sec]	Mass fragment fingerprint (m/z)	Name of compound and molecular formula	Class of compound	MW (Da)
1	17.8712	5730418.12	79, 91, 108, 133, 148, 216	Benzofuran,6-ethenyl-4,5,6,7-tetrahydro-3,6-dimethyl-5-isopropenyl-,trans- ($C_{15}H_{20}O$)	Sesquiterpene	216
2	20.0827	55396058.51	39, 66, 91, 94, 122, 123, 162, 215, 230	Cycloisolongifolence,8,9-dehydro-9-formyl- ($C_{16}H_{22}O$)	Sesquiterpene	230
3	20.4091	11318359.69	41, 55, 69, 81, 93, 107, 121, 136, 147, 193, 236	7a-Isopropenyl-4,5-dimethyloctahydroindene-4-carboxylic acid ($C_{15}H_{24}O_2$)	Sesquiterpene	236
4	21.7946	4285329.96	41, 53, 67, 79, 91, 107, 121, 135, 136, 147, 175, 203, 218	3,7-Cyclodecadien-1-one,3,7-dimethyl-10-(1-methylethylidene),(E,E)- ($C_{15}H_{22}O$)	Sesquiterpene	218
5	22.2343	38927338.22	41, 55, 68, 82, 95, 109, 121, 139, 165, 167, 180, 181	6,10-Dimethyl-3-(1-methylethyl)-6-cyclodecene-1,4-dione ($C_{15}H_{24}O_2$)	Sesquiterpene	236
6	22.4874	18038776.21	41, 55, 67, 79, 91, 105, 119, 133, 147, 165, 189, 196, 219, 234	Tricyclo[5.1.0.0(2,4)]oct-5-ene-5-propanoic acid,3,3,8.8-tetramethyl- ($C_{15}H_{22}O_2$)	Sesquiterpene	234
7	22.8471	6525373.55	41, 68, 69, 82, 95, 109, 121, 139, 165, 167, 180, 181	Neocurdione ($C_{15}H_{24}O_2$)	Sesquiterpene	236
8	23.4133	11801673.62	41, 67, 68, 81, 96, 109, 122, 164, 165, 178, 234	2(3H)-Benzofuranone,6-ethenylhexahydro-3,6-dimethyl-7-(1-methylethenyl)-,[3S-(3α,3aα,6α,7β,7aβ)] ($C_{15}H_{22}O_2$)	Sesquiterpene	234
9	23.5532	10695867.24	41, 53, 65, 79, 91, 108, 135, 162, 163, 189, 203, 232, 233	5,6-Azulenedicarboxaldehyde,1,2,3,3a,8,8a-hexahydro-2,2,8-trimethyl-,(3aα,8α,8aα)-(-)- ($C_{15}H_{20}O_2$)	Aromatic compound	232
10	23.7464	4306170.71	41, 53, 77, 81, 93, 107, 122, 123, 150, 162, 215, 230	Azuleno[4,5-b]furan-2(3H)-one,3a,4,6a,7,8,9,9a,9b-octahydro-6-methyl-3,9-bis(methylene),[3aS-(3aα,6aα, 9aα,9bβ)]- ($C_{15}H_{18}O_2$)	Fused Aromatic compound	230
11	23.9795	1968432.46	43, 44, 67, 93, 105, 121, 136, 147, 159, 191, 201, 232	Isoaromadendrene epoxide ($C_{15}H_{24}O$)	Sesquiterpene	220
12	24.4658	45277656.11	41, 67, 68, 69, 107, 121, 167	4-Oxo-β-isodamascol ($C_{13}H_{20}O_2$)	Substituted cyclic ketone	208

13	26.9238	3707821.96	43, 44, 67, 79, 91, 105, 123, 147, 173, 201, 216, 217	6-(1-Hydroxymethylvinyl)-4,8a-dimethyl-3,5,6,7,8,8a-hexahydro-1H-naphthalene-2-one ($C_{15}H_{22}O_2$)	Sesquiterpene	234
14	27.1835	25623614.92	43, 53, 65, 77, 91, 105, 119, 122, 147, 161, 175, 188, 246	2H-Cyclohepta[b]furan-2-one,6-[1-(acetyloxy)-3-oxobutyl]-3,3a,4,7,8,8a-hexahydro-7-methyl-3-methylene- ($C_{17}H_{22}O_5$)	Fused heterocyclic compound	306
15	32.1528	2454757.59	69, 81, 105, 123, 137, 147, 177, 199, 207, 228, 273, 302	4,8,13-Cyclotetradecatriene-1,3-diol,1,5,9-trimethyl-12-(1-methylethyl)- ($C_{20}H_{34}O_2$)	Substituted cyclic hydrocarbon	306
16	33.3718	735576.90	69, 97, 109, 117, 147, 161, 175, 191, 207, 239, 255, 280, 340, 407	9,12,15-Octadecatrienoic acid-2,3-bis(acetyloxy)propyl ester,[Z,Z,Z]- ($C_{25}H_{40}O_6$)	unsaturated fatty acid	436

detected exclusively in CC-P extract (Table 1). On the other hand, Phenol,2,4-bis (1,1-dimethylethyl)-; 2-(4a,8-Dimethyl-6-oxo-1,2,3,4-4a,5,6,8a-octahydro-naphthalen-2-yl) propionaldehyde; 4,8a-Dimethyl-6-(2-methyl-oxiran-2-yl)-4a,5,6,7,8,8a-hexahydro-1H-naphthalen-2-one; 2-[4-Methyl-6-(2,6,6-trimethylcyclohex-1-enyl]hexa-1,3,5-trienyl] cyclohex-1-en-1-carboxaldehyde; Androstan-3-one,17-hydroxy-1,17-dimethyl, (1α,5α,17β)-; 2-Propenal,3-(2,6,6-trimethyl-1-cyclohexen-1-yl)-; 2H-cyclohepta[b]furan-2-one,6-[1-(acetyloxy)-3-oxobutyl]-3,3a,4,7,8,8a-hexahydro-7-methyl-3-methylene-; Dihydroxanthin; Stigmastane-3,6-dione, (5α)- and β-Sitosterol were exclusively detected in CC-C extract (Table 2**)**. The abundance of a particular compound (%) was calculated with respect to area (intensity height x time) of corresponding peak relative to total area of entire chromatogram including both identified and unidentified peaks. Accordingly, the major constituents (relative abundance > 4%) present in CC-P were cycloisolongifolence, 8,9-dehydro-9-formyl- (19.64%), 4-oxo-β-isodamascol (16.05%), 6,10-dimethyl-3-(1-methylethyl)-6-cyclodecene-1,4-dione (13.80%), 2H-cyclohepta[b] furan-2-one,6-[1-(acetyloxy)-3-oxobutyl]-3,3a,4,7,8,8a-hexahydro-7-methyl-3-methylene- (9.08%), tricycle [5.1.0.0(2,4)] oct-5-ene-5-propanoic acid,3,3,8.8-tetramethyl- (6.39%), 2 (3H)-benzofuranone,6-ethenylhexahydro-3,6-dimethyl-7-(1-methylethenyl)-, [3S-(3α,3aα,6α,7β,7aβ) (4.18%) and 7a-isopropenyl-4,5-dimethyloctahydroindene-4-carboxylic acid (4.01%). Similarly, the major constituents present in CC-C were cycloisolongifolence, 8,9-dehydro-9-formyl-(15.69%), 6,10-dimethyl-3-(1-methylethyl)-6-cyclodecene-1,4-dione (13.38%), 2H-cyclohepta[b]furan-2-one,6-[1-(acetyloxy)-3-oxobutyl]-3,3a,4,7,8,8a-hexahydro-7-methyl-3-methylene- (4.55%), tricycle [5.1.0.0(2,4)] oct-5-ene-5-propanoic acid,3,3,8.8-tetramethyl- (7.50%), 2(3H)-benzofuranone,6-ethenylhexahydro-3,6-dimethyl-7-(1-methylethenyl)-, [3S-(3α,3aα,6α,7β,7aβ) (6.15%), 7a-isopropenyl-4,5-dimethyloctahydroindene-4-carboxylic acid (6.43%) and 2-(4a,8-Dimethyl-6-oxo-1,2,3,4-4a,5,6,8a-octahydro-naphthalen-2-yl) propionaldehyde (6.57%). The list of the major constituents present in CC-P and CC-C extracts and their known bioactivities is given below.

i) Cycloisolongifolence,8,9-dehydro-9-formyl- with 19.64% relative abundance (RA). It has antitumor, anti-plasmodial, and anti-inflammatory activities (Lai *et al*. 2044; Lakshmi *et al*. 2011; Chadwick *et al*. 2013; Rita de Cássia *et al*. 2015)

Table 2. GC-MS identification of phytochemicals present in chloroform extract (CC-C) of the rhizome of *C. caesia*

Sl. No.	RT	Area [Intens sec]	Mass fragment fingerprint (m/z)	Name of compound and molecular formula	Class of compound	MW (Da)
1	14.7525	11903149.65	41, 79, 91, 108, 133, 148, 216	Benzofuran,6-ethenyl-4,5,6,7-tetrahydro-3,6-dimethyl-5-isopropenyl-trans- ($C_{15}H_{20}O$)	Sesquiterpene	216
2	15.0057	3805507.39	57, 74, 163, 191, 206	Phenol,2,4-bis(1,1-dimethylethyl)- $C_{14}H_{22}O$	Phenolic compound	206
3	16.4312	70809565.21	39, 66, 91, 94, 122, 123, 162, 215, 230	Cycloisolongifolene,8,9-dehydro-9-formyl- ($C_{16}H_{22}O$)	Sesquiterpene	230
4	16.7176	29040931.29	41, 55, 69, 93, 107, 121, 136, 147, 193, 236	7a-Isopropenyl-4,5-dimethyloctahydroindene-4-carboxylic acid ($C_{15}H_{24}O_2$)	Sesquiterpene	236
5	17.7501	9953906.77	41, 53, 67, 79, 91, 107, 121, 135, 136, 147, 175, 218	3,7-Cyclodecadien-1-one,3,7-dimethyl-10-(1-methylethylidene),(E,E)- ($C_{15}H_{22}O$)	Germacrone	218
6	18.0898	60415463.56	41, 55, 69, 82, 95, 109, 121, 139, 165, 167, 180, 181	6,10-Dimethyl-3-(1-methylethyl)-6-cyclodecene-1,4-dione ($C_{15}H_{24}O_2$)	Sesquiterpene	236
7	18.2897	33944990.51	41, 55, 67, 79, 91, 105, 133, 165, 189, 191, 219, 234	Tricyclo[5.1.0.0(2,4)]oct-5-ene-5-propanoic acid,3,3,8.8-tetramethyl- ($C_{15}H_{22}O_2$)	Sesquiterpene	234
8	18.5495	10241547.94	41, 68, 69, 82, 95, 109, 121, 139, 165, 167, 180, 181	Neocurdione ($C_{15}H_{24}O_2$)	Sesquiterpene	236
9	18.9958	29404606.41	41, 55, 67, 68, 95, 96, 109, 121, 152, 164, 178, 191, 219, 234	2(3H)-Benzofuranone,6-ethenylhexahydro-3,6-dimethyl-7-(1-methylethenyl)-,[3S-(3α,3aα,6α,7β,7aβ)] ($C_{15}H_{22}O_2$)	Sesquiterpene	234
10	19.2622	5417179.54	41, 55, 77, 81, 94, 107, 122, 123, 150, 162, 215, 230	Azuleno[4,5-b]furan-2(3H)-one,3a,4,6a,7,8,9,9a,9b-octahydro-6-methyl-3,9-bis(methylene),[3aS-(3aα,6aα, 9aα,9bβ)]- ($C_{15}H_{18}O_2$)	Fused aromatic compound	230
11	19.7285	29680039.62	43, 53, 68, 79, 91, 107, 133, 161, 176, 177, 219, 234	2-(4a,8-Dimethyl-6-oxo-1,2,3,4-4a,5,6,8a-octahydro-naphthalen-2-yl) propionaldehyde ($C_{15}H_{22}O_2$)	Sesquiterpene	234
12	20.6478	7850033.86	43, 55, 67, 79, 91, 105, 121, 133, 161, 163, 173, 197, 212, 234	6-(1-Hydroxymethylvinyl)-4,8a-dimethyl-3,5,6,7,8,8a-hexahydro-1H-naphthalene-2-one ($C_{15}H_{22}O_2$)	Sesquiterpene	234

13	22.2265	20562711.13	43, 53, 65, 77, 91, 105, 119, 123, 147, 161, 175, 188, 203, 246	2H-Cyclohepta(b)furan-2-one,6-[1-(acetyloxy)-3-oxobutyl]-3,3a,4,7,8,8a-hexahydro-7-methyl-3-methylene- ($C_{17}H_{22}O_5$)	Xanthinin	306
14	22.5195	12907768.89	43, 55, 67, 81, 107, 122, 149, 173, 191, 206, 219	4,8a-Dimethyl-6-(2-methyl-oxiran-2-yl)-4a,5,6,7,8,8a-hexahydro-1H-naphthalen-2-one ($C_{15}H_{22}O_2$)	Sesquiterpene	234
15	22.9259	6772960.15	43, 55, 67, 81, 107, 123, 136, 148, 191, 219	2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl]hexa-1,3,5-trienyl]cyclohex-1-en-1-carboxaldehyde ($C_{23}H_{32}O$)	Unsaturated substituted hydrocarbon	324
16	23.7519	3562571.26	43,55, 67, 79, 91, 105, 131, 147, 161, 174, 175, 189	Androstan-3-one,17-hydroxy-1,17-dimethyl,(1,5α,17β)- ($C_{21}H_{34}O_2$)	Steroid	318
17	23.9650	5080956.50	43, 69, 81, 91, 105, 145, 175, 189, 213, 228, 246, 247	2-Propenal,3-(2,6,6-trimethyl-1-cyclohexen-1-yl)- ($C_{12}H_{18}O$)	Ionone isomer (terpene)	178
18	27.9951	7112246.57	43, 55, 67, 81, 95, 110, 124, 147, 159, 190, 205, 230, 248	Dihydroxanthin ($C_{17}H_{24}O_5$)	Terpene	308
19	37.6206	16989684.90	43, 69, 81, 95, 107, 137, 147, 175, 189, 207, 217, 231, 245, 246, 247, 287, 288, 316, 331, 359, 385, 399, 413, 426	Stigmastane-3,6-dione,(5α)- ($C_{29}H_{48}O_2$)	Steroid	428
20	44.1753	2901572.24	55, 81, 95, 107, 145, 161, 173, 199, 207, 213, 239, 255, 273, 281, 303, 329, 355, 381, 396, 414	β-Sitosterol ($C_{29}H_{50}O$)	Sterol	414

ii) 4-Oxo-β-isodamascol with 16.05 % RA.

iii) 6,10-Dimethyl-3-(1-methylethyl)-6-cyclodecene-1,4-dione with 13.80 % RA

iv) 2H-Cyclohepta[b]furan-2-one,6-[1-(acetyloxy)-3-oxobutyl]-3,3a,4,7,8,8a-hexahydro-7-methyl-3-methylene- with 9.08 % RA.

v) Tricyclo [5.1.0.0(2,4)] oct-5-ene-5-propanoic acid,3,3,8.8-tetramethyl with 6.39 % RA. It has anticancer, Anti-plasmodial, and Anti-inflammatory activities (Chadwick *et al*. 2013; Rita de Cássia*et al*. 2015.

vi) 2 (3H)-Benzofuranone,6-ethenylhexahydro-3,6-dimethyl-7-(1-methylethenyl)-,[3S-(3α,3aα,6α,7β,7aβ with 4.18 % RA.

vii) 7a-Isopropenyl-4,5-dimethy-loctahydroindene-4-carboxylic acid with 4.01 % RA.

Componds iii,v,vi,vii also have anticancer, antiplasmodial, and antiinflammatory activities (Chadwick *et al*. 2013; Rita de Cássia *et al*. 2015).

Major putative phytoconstituents identified by GC-MS in chloroform extract (CC-C) of the rhizome of *C. caesia* are

i.) Cycloisolongifolence,8,9-dehydro-9-formyl- with 15.69% RA.

ii) 6,10-Dimethyl-3-(1-methylethyl)-6-cyclodecene-1,4-dione with 13.38% RA

iii) 2H-Cyclohepta[b]furan-2-one,6-[1-(acetyloxy)-3-oxobutyl]-3,3a,4,7,8,8a-hexahydro-7-methyl-3-methylene- with 4.55% RA

iv) Tricyclo [5.1.0.0(2,4)] oct-5-ene-5-propanoic acid,3,3,8.8-tetramethyl- with 7.50% RA

v) 2(3H)-Benzofuranone, 6-ethenylhexahydro-3,6-dimethyl-7-(1-methylethenyl)-,[3S-(3α,3aα,6α,7β,7aβ) with 6.51% RA

vi) 7a-Isopropenyl-4,5-dimethyloctahydroindene-4-carboxylic acid with 6.43% RA

vii) 2-(4a,8-Dimethyl-6-oxo-1,2,3,4-4a,5,6,8a-octahydro-naphthalen-2-yl) propional dehyde with 6.57% RA.

All the compounds except compound (iii) have anti-cancer, anti-plasmodial, and anti-inflammatory activities (Chadwick *et al*. 2013; Rita de Cássia *et al*. 2015).

Previous studies aimed at identifying phytochemicals from the essential oil of rhizome of *C. caesia* have indicated the predominance of sesquiterpenes, camphene, borylene, and δ-camphor classes of compounds (Sastri 1962). However, the present study for the first time have compared GCMS profiling of *C. caesia* rhizome extracts of petroleum ether and chloroform and the major compounds present in these extracts were sesquiterpenes.

Biocidal actions of extracts

The results of antibacterial assay are shown in Table 3**.** Both the CC-C and CC-P showed inhibitory effect against all the bacterial test pathogens used in this study. The inhibition zone ranged from 10 mm to 18.25 mm. In this study, *Micrococcus luteus* showed the highest sensitivity against CC-C and CC-P extracts with the nearly similar zone of inhibition of ~18 mm whereas *Bacillus subtilis* showed the lowest sensitivity against both the extracts. The difference in the zone of inhibition against various pathogens might be due to the diffusing potency of the extract against the cell wall of a particular bacterium.

Further, the results of antifungal assay are shown in Table 4. Both the extracts (CC-C and CC-P) were found to show antifungal activity against *Rhizoctonia solani* with the nearly similar zone of inhibition of ~ 10 mm but failed to show activity against *Aspergillus niger*. Above results thus suggested that the extracts exhibited broad spectrum activity against the pathogenic bacteria. The phytoconstituents such as flavonoids, and polyphenolic compounds are characteristically reported for contributing medicinal activities such as anti-oxidant, anti-allergic, antimicrobial, anti-inflammatory and anti-cancer (Aiyelaagbe & O samudiamen 2009). Similarly, anti-microbial activity of plant extract is known to be contributed by phytochemicals such as alkaloids, flavonoids, saponins, tannins, and steroids (Nethathe & Ndip 2011). Since phytochemical screening of the CC-P and CC-C extracts confirmed the presence of all the above classes of chemicals, it justified their bioactivities.

Evaluation of antioxidant activity

Neutralization of ABTS and DPPH radicals

The outcomes of ABTS and DPPH assays suggested that the optical density (OD) of ABTS/DPPH solution decreased in the presence of the increasing concentrations of the extracts (CC-P/CC-C). This suggested the efficacy of extracts for neutralizing of ABTS and DPPH as antioxidants. The percentage (%) neutralization or scavenging calculated from the decrease in absorbance of DPPH/ ABTS solution are shown in figures 2 A & B respectively. From these graphs, the IC_{50} values for DPPH neutralization (concentration of extract that reduced the OD of DPPH solution to half of its initial value) estimated was 0.68±0.02

Table 3. Antibacterial activity of the chloroform (CC-C) and petroleum ether (CC-P) extracts of the rhizomes of *C. caesia*. Values are mean of duplicate readings (mean ± SD)

Sl. No	Crude extract	Concentration (μg mL^{-1})	Zone of inhibition (in mm)			
			Bacillus subtilis	*Micrococcus luteus*	*Escherichia coli*	*Mycobacterium smegmatis*
1	CC-C	25	10 ± 0	18 ± 0	16.75 ± 0.75	14.5 ± 0.5
2	CC-P	25	12.5 ± 0.5	18.25 ± 0.25	13 ± 0	15 ± 0
3	Ampicillin	25	25 ± 0	30.5 ± 0.5	28 ± 1	24 ± 0

Table 4. Antifungal activity of the chloroform (CC-C) and petroleum ether (CC-P) extracts of the rhizomes of *C. caesia*. Values are mean of duplicate readings (mean ± SD)

Sl. No	Crude extract	Concentration (μg mL^{-1})	Zone of inhibition (in mm)	
			Rhizoctonia solani	*Aspergillus niger*
1	CC-C	25	10.5±0.5	-
2	CC-P	25	10.5±0.5	-
3	Fluconazole	25	20.5±0.75	17.5±0.5

mg mL^{-1} and 0.13±0.01 mg mL^{-1} for CC-P and CC-C extracts, respectively (inset of Fig. 2A). Similarly, IC_{50} values of ABTS neutralization was 58.35±3.50 and 36.60±2.00 μg mL^{-1} for CC-P and CC-C, respectively (inset of Fig. 2 B). The observed results revealed that the scavenging activity of CC-C extract for DPPH and ABTS radicals was ~5 times and ~1.5 times higher as compared to the CC-P extract.

Inhibition of lipid peroxidation

The γ-irradiation of the aqueous solution of liposomes facilitates oxidation of the lipid molecules through radiolytically generated hydroxyl radicals and forms malondialdehyde (MDA) as one of the final products of lipid per oxidation. The liposomal solution treated with a fixed concentration (50 μg mL^{-1}) of CC-P and CC-C extracts prior to γ-radiation showed

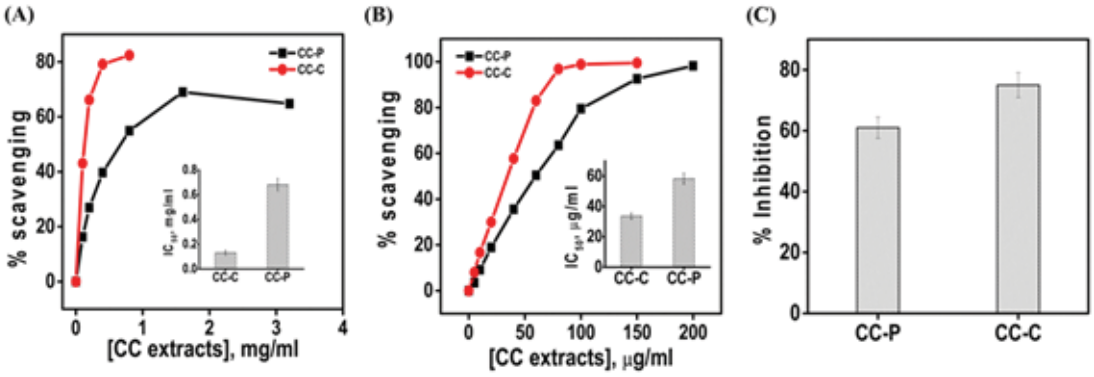

Fig. 2 (A) & (B) Plots of percentage (%) scavenging of DPPH and ABTS radical respectively vs concentration of extract. Inset of (A) and (B) show the IC_{50} values of extract obtained from DPPH and ABTS assays. (C) Plot of percentage (%) inhibition of lipid peroxidation at fixed concentration (50 μg ml^{-1}) of CC-P and CC-C extracts. Values are mean of duplicate readings (mean ± SD).

decrease in the MDA content measured as TBARS as compared to untreated (liposomal solution without CC extracts) radiation control. The % inhibition of lipid peroxidation calculated in terms of the decrease in the TBARS content was 61.0±3.5% and 75.0±4.1% respectively for CC-P and CC-C extracts (Fig. 2 C). This result suggested that CC-C extract was better than CC-P for inhibiting the lipid peroxidation which is in line with the results of DPPH and ABTS assays. The difference in the antioxidant activities of CC-P and CC-C extracts is attributed to the differential solubility of phytoconstituents between the polar and non-polar solvents.

Conclusion

The study documented 16 major volatile compounds in CC-P and 20 compounds in CC-C extracts. The extracts showed similar antibacterial and antifungal activities, but differed in anti-oxidant activities.

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