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Original article

The potential of antioxidant activity of methanolic extract of *Coscinium fenestratum* (Goetgh.) Colebr (Menispermaceae)

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ABSTRACT

To explore the possible bioactive compounds and to study the antioxidant capacity of *Coscinium fenestratum* (Goetgh.) Colebr (Menispermaceae), the qualitative and quantitative phytochemical screening for various secondary metabolites were evaluated. Using the GC–MS analysis, a total number of 30 phytochemical compounds were predicted with their retention time, molecular weight, molecular formula, peak area, structure and activities. The most prevailing heterocyclic compound was Bis(2,4,6- triisopropylphenyl) phosphinicazide (6.70%). The antioxidant activity was evaluated by spectrophotometric methods using the reducing power assay and the DPPH· and ABTS·+ scavenging assays. The activity was determined to be increased in all the test samples with the increase in the volume of the extract. *C. fenestratum* possess a good source of many bioactive compounds that are used to prevent diseases linked with oxidative stress.

1. Introduction

The natural products from plant origin are safer than the synthetic drug molecules, and are widely recognized in the pharmaceutical industries for their broad structural diversity and the pharmacological activities (Newman and Cragg, 2016; Thenmozhi et al., 2018). *Coscinium fenestratum* (Goetgh.) Colebr. (-commonly known as 'tree turmeric', belongs to the family Menispermaceae), is a medicinally important dioecious threatened liana (Tushar et al., 2008), distributed in Vietnam, Singapore, Thailand, Sri Lanka and in isolated regions of the Western Ghats of India (Ved et al., 2015). The stem and root of *C. fenestratum* are used in the traditional system of medicine (Tushar et al., 2008). The active chemical berberine (-a natural isoquinoline alkaloid), ceryl alcohol, hentriacontane, palmitic acid, sitosterol, saponin with some resinous material and oleic acid have earlier been reported from *C. fenestratum* (Rojsanga et al., 2006) which possess variety of pharmacological activities including antidiabetic, anti-inflammatory, thermogenic and antiseptic activity (Kashyap et al., 2016). The free radicals and the other reactive oxygen species (ROS) generated within the living cells as a result of physiological and biochemical processes of the cells causes oxidative damage to the macromolecules of the cells, which lead to liver diseases (Arteel, 2003), asthma (Lobo et al., 2010; Bharathi et al., 2018), cancer (Kinnula and Crapo, 2004), chronic inflammation, diabetes, multiple sclerosis (Lobo et al., 2010; Bharathi et al., 2018), neural disorders (Sas et al., 2007), rheumatoid arthritis (Lobo et al., 2010; Bharathi et al., 2018), cardiovascular disease (Singh and Jialal, 2006), Alzheimer disease (Smith et al., 2000), Parkinson's disease (Bolton et al., 2000), ulcerative colitis (Ramakrishna et al., 1997), and aging (Hyun et al., 2006). The free radicals and other reactive oxygen species can be scavenged by the protective role of antioxidants from the natural products of wild and medicinal plants (Pietta et al., 1998). Hence, the objective of the present study was to investigate the phytochemical constituents and antioxidant activity of *C. fenestratum*.

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2. Materials and methods

2.1. Collection of the plant sample and the preparation of methanolic crude extract

The fresh leaves of *C. fenestratum* were collected from Velliangiri hills of Western Ghats, Coimbatore, Tamil Nadu, India. The semidry methanolic crude extract [MeOHCf, test compound] was prepared from 50 g of shade dried powdered leaves using soxhlet extractor.

2.2. In vitro antioxidant activity

There are various *in vitro* and *in vivo* methods available for the evaluation of the antioxidant activity of natural products (Alam et al., 2013). The reducing ability (Yildirim et al., 2001), DPPH radical scavenging activity (Blois, 1958), the total antioxidant activity (Siddhuraju and Manian, 2007) of MeOHCf were determined using the standard method in order to evaluate the *in vitro* antioxidant activity. One way analysis of variance (ANOVA) test was carried out for statistical analysis using SPSS 10.0.

2.3. Identification of the phytochemical components of MeOHCf by GC–MS analysis

The qualitative phytochemical analysis of MeOHCf for the phytochemicals *viz.,* alkaloids, cardiac glycosides, glycosides, flavonoids, phenols, resins, steroids, saponins, tannins, triterpenoids terpenoids, were performed (Harbone, 1973; Trease and Evans, 1983). The alkaloid contents of MeOHCf was determined gravimetrically (Obadoni and Ochuko, 2001). The total phenolic and tannin contents of MeOHCf were estimated, and it was expressed as gallic acid equivalents (GAE) mg/g extract (Siddhuraju and Becker, 2003). The content of total flavonoids was determined spectrophotometrically using a standard curve rutin (Zhishen et al., 1999). MeOHCf was then subjected to the gas chromatography–mass spectrometry (GC–MS) analysis using 5975C Agilent Technologies GC systems equipped with DB-5 ms Agilent fused silica capillary column (30 × 0.25 mm ID, 0.25 μm film thickness) operating with electron impact mode at 70 eV. Finally MeOHCf was assigned for comparison of their retention indices and the mass spectra fragmentation patterns with chemical library of NIST (National Institute of Standards and Technology).

3. Results and discussion

The percentage yield of MeOHCf was 15.8% w/w. The preliminary phytochemical screening of MeOHCf revealed the presence of flavonoids, saponins and steroids in maximum amount. In addition, the chemical constituents such as alkaloids, terpenoids, triterpenoids, glycosides, cardiac glycosides, phenols, tannins and resins were also present (Table 1). Alkaloids have medicinal importance for the treatment of cancer, CNS disorders, hypertension, inflammation, and malaria (Patel et al., 2012). Phenols have been reported to possess the properties of quenching of oxygen-derived free radicals (Oksana et al., 2012). Tannins have been reported to possess a strong astringent properties as well as antimicrobial, anti-inflammatory and antioxidant activity, causing protein precipitation (Tyler et al., 1988). Flavonoids increases the capillary permeability and have been used for the treatment of cardiovascular diseases and possess potential antioxidant and anti-inflammatory properties (Das and Pereira, 1990).

The spectrum profile of GC–MS was confirmed the presence of a total number of thirty compounds in MeOHCf (Table 2). The prevailing compounds [the total running time: 40.16 min. (Fig. 1)] were Bis(2,4,6-triisopropylphenyl) phosphinicazide, a hetrocyclic compound (peak area: 6.70%) (Fig. 2a), Methyl 2-N-cyclohexyla mino-2,3-dideoxy-4,6-o-(phenylmethylene)-3-c-phenylsulfonyl-à, D-glucopyranoside (peak area: 5.99%) (Fig. 2b), Cyclohexane, 1,4-dimethyl-2-octadecyl- (CAS), an alcohol (peak area: 5.82) (Fig. 2c) and à-Cyperone, a sesquiterpene (peak area: 5.81) (Fig. 2d). *C. fenestratum*is endowed with various medicinal properties. Among the identified compounds, EthylN-(p-tolylsulfinyl) (à-trifluoromethyl)-à-allylglycinate was reported to exert antiproliferative and antitumor activities (Leonardo et al., 2014). Anti-inflammatory property was shown by compounds 2-Thienyl methylo-(3′-<t-butyl > amino-2′-hydroxypropyl) ketoxime, Ethyl N-benzylanthranilate, à-Cyperone and Pyranthrene (Al-Riyami et al., 2013). Two compounds *viz.*, Cyclohexane, 1,4-dimethyl-2-octadecyl- (CAS) and 10-[(3′,5′-Bis(trifluoromethyl)phenyl]-3-(eth oxycarbonyl methyl)ioalloxazine are reported to have anticancer property (Jungwirth et al., 2012). Aromatic heteropolycyclic compound, 17-(Cyclopropylmethyl)-à-(1′,1′-dimethylethyl) -4,5-epoxy -18,19-dihydro -3-hydroxy -6-methoxy-à-methyl -6,14-ethenomorphinane-7-methanol is a strong opioid antagonist, used as a tranquilizing agent in veterinary medicine (Furst et al., 1995). The compound, 1,5,6, 10b-Tetrahydro-8,9-dimethoxy-10b-(p-met hoxyphenyl)-2-methylene- 2H-isoxazolo [3,2-a] isoquinoline-1-carbonitrile is used to lower the blood pressure and acts as antihypertensive agent (Peacock et al., 2015). In addition, various traditional medicinal plant species of Menispermaceae have been analyzed phytochemically using GC–MS, and suggested for drug preparation after succeeding clinical trials (Chandra and Lakshmi, 2015; Ramesh et al., 2016).

Table 1
Percentage yield and qualitative phytochemical analysis of MeOHCf.

Phytochemical constituents	Trace	Qualitative phytochemical analysis
Yield (%)	15.8	–
Alkaloids	++	Total alkaloids (mg/g of dry powder): 52.00 ± 0.19
Flavonoids	+++	Total flavonoids (mg of QE/g extract): 42.01 ± 0.06
Terpenoids	++	–
Triterpenoids	++	–
Glycosides	+	–
Cardiac glycosides	+	–
Phenols	++	Total phenols (mg of GAE/g extract): 35.11 ± 0.04
Saponins	+++	–
Steroids	+++	–
Tannins	++	Total tannins (mg of GAE/g extract): 34.46 ± 0.02
Resins	+	–

+: Present, ++: Moderately present, +++: Highly present, GAE: Gallic Acid Equivalent, QE: Quercetin Equivalent. Values were performed in triplicates and represented as mean ± SD.

The antioxidants molecules helps in preventing diseases by neutralize the effects of ROS (Sindhi et al., 2013). The antioxidant property of MeOHCf was determined using various methods. In reducing power assay, MeOHCf displayed significant activity which was found to increase with the increase in the concentration (Table 3) which may serve as significant indicator for the potential antioxidant activity. The results of the resent study were in accordance with the previous reports (Karthika et al., 2014; Thenmozhi et al., 2015). The percentage of scavenging activity on the DPPH radical varies from 32.54% (50 μg/mL of extract) to 64.80% (250 μg/mL of extract). The IC_{50} value of MeOHCf was 182.48 μg/mL (Table 4). The extract showed significant scavenging effect on the DPPH· which was increasing with the increase in the concentration of the sample from 50 to 250 μg/mL, which might be due to abundance of the flavonoid (42 mg of QE/g extract) content, the most

Table 2
Compounds identified in the MeOHCf by GC–MS.

S. no.	Name of the compound	RT	Molecular formula	Molecular weight	Peak area (%)	Category of the compound	Activity[a]
1.	EthylN-(p-tolylsulfinyl)(à-trifluoromethyl)-à-allylglycinate	4.16	$C_{15}H_{18}F_3NO_3S$	349	2.56	Cyclic compound	Antiproliferative and antitumor properties
2.	Trimethylester of(4r,5s:4s,5r)-5-(methoxycarbonylmethyl)-1-methyl-2-pyrazolin-3,4,5-tricarbxylic acid	4.98	$C_{13}H_{18}N_2O_8$	330	1.76	Hetrocyclic compound	No activity reported
3.	2-Thienylmethylo-(3′-<t-butyl > amino-2′-hydroxypropyl) ketoxime	12.60	$C_{14}H_{23}N_2O_2S$	283	1.74	Hetrocyclic compound	Antiinflammatory activity
4.	Benzaldehyde, 4-hydroxy-3-methoxy-(CAS)	14.11	$C_8H_8O_3$	152	2.12	Phenolic aldehyde	Anticonvulsant, antioxidant, antimutagenic agents
5.	D-friedoolean-14-en-3-one (CAS)	21.72	$C_{30}H_{48}O$	424	2.41	Triterpenoid derivatives	Antifungal and antioxidant agents
6.	Ethyl N-benzylanthranilate	22.03	$C_{16}H_{17}NO_2$	255	2.55	Coumarin	Antiinflammatory activity
7.	(E)-á-[2-hydroxyphenylethylene]benzeneethanol-D2	23.38	$C_{15}H_{12}D_2O_2$	226	1.64	–	No activity reported
8.	Himachalol	25.23	$C_{15}H_{26}O$	222	2.63	Sesquiterpene alcohol	Insecticidal activity, Antitumor activity
9.	1,5,6, 10b-Tetrahydro-8,9-dimethoxy-10b-(p-methoxyphenyl)-2-methylene-2H-isoxazolo[3,2-a]isoquinoli ne-1-carbonitrile	25.64	$C_{22}H_{22}N_2O_4$	378	1.54	Organic compound	Lowers blood pressure, Antihypertensive agent
10.	1,9-Dimethoxy-10-methyl-2-(carbamoylmethylcarbonyl)-3-(methoxycarbonylmethyl)-10-methyl-anthracene	26.15	$C_{23}H_{23}NO_6$	409	2.82	Alkaloid	Immunosuppressive agent
11.	à-Cyperone	26.78	$C_{15}H_{22}O$	218	5.81	Sesquiterpene	Antiinflammatory activity
12.	17-(Cyclopropylmethyl)-à-(1′,1′-dimethylethyl)-4,5- epoxy-18,19-dihydro-3-hydroxy-6-methoxy-à-methyl -6,14-ethenomorphinane-7-methanol	27.19	$C_{26}H_{35}NO_4$	425	1.68	Aromatic heteropolycyclic compound	Strongest opioid antagonist and used for tranquilizing large animals in veterinary medicine.
13.	1-P-menthen-8-yl acetate	27.74	$C_{12}H_{20}O_2$	196	1.76	Aromatic compound	Flavor and fragrance agent
14.	6-Bromohexanoic acid, 10-undecenyl ester	28.51	$C_{17}H_{31}BrO_2$	346	2.54	Aliphatic compound	No activity reported
15.	2-[Diacetylamino]-6-(3′-methyl-5′-oxo-1′-phenyl-2′-pyrazolin-4′-yl)-4-phenylpyridine-3-carbonitrile	30.32	$C_{26}H_{21}N_5O_3$	451	4.80	Aliphatic compound	No activity reported
16.	Pyranthrene	31.02	$C_{30}H_{16}$	376	5.16	Aromatic compound	Antiinflammatory activity
17.	Methyl 2-N-cyclohexylamino-2,3-dideoxy-4,6-o-(phenylme thylene)-3-c-phenylsulfonyl-à,D-glucopyranoside	31.73	$C_{26}H_{32}NO_6S$	486	5.99	–	No activity reported
18.	1-Pyrrolidino-benzoanthra-9,10-quinnone	32.03	$C_{22}H_{21}NO_2$	331	2.95	Heterocyclic aromatic organic compound	Antibiotic agent
19.	(22E)-3áAcetoxy- 7.alpha,-hydroperoxystigmasta-5, 22-diene	32.69	$C_{31}H_{50}O_4$	486	1.54	Triterpene	Piscicidal activity
20.	1-Diphenylphosphino-1-dichlorophosphino-[1]-ferrocene	33.34	$C_{22}H_{18}C_{12}FeP_2$	470	4.24	Organophosphorus	No activity reported
21.	Bis(2,4,6-triisopropylphenyl)phosphinicazide	35.54	$C_{30}H_{46}N_3OP$	495	6.70	Hetrocyclic compound	Antitumor and antifolate properties
22.	6-[N-(Cyanoamino)]-3á-methoxymethoxy-cholestane	36.62	$C_{30}H_{50}N_2O_2$	470	3.85	Alkaloid	Antibiotic agent
23.	2,3-Bis(3′,4′-dimethoxyphenyl)-5,6-difluorobenzo[b]Furan	36.96	$C_{24}H_{20}F_2O_5$	426	4.02	Aromatic compound	Antidepressant
24.	5á-Androst-16-en-3à-ol-[(t-butyl)dimethylsilyl] ether	37.43	$C_{25}H_{44}OSi$	388	2.88	Aromatic compound	Antidepressant
25.	3-(4-Chlorobenzoyl)-7-methyl-2-(2-methylphenylimino)indole	38.00	$C_{23}H_{19}C_1N_2O$	374	4.62	Organochlorine	Antifungal and antioxidant properties
26.	Cyclohexane, 1,4-dimethyl-2-octadecyl- (CAS)	38.37	$C_{26}H_{52}$	364	5.82	Alcohol	Anticancer agent
27.	10-[(3′,5′-Bis(trifluoromethyl)phenyl]-3-(ethoxycarbonylmethyl)ioalloxazine	38.61	$C_{22}H_{14}F_6N_4O_4$	512	2.20	Tricyclic compound	Anticancer agent
28.	13-Docosenamide, (z)-	39.00	$C_{22}H_{43}NO$	337	4.46	Carboxylic acid amide	Used as a detergent, fabric softener, anti-static agent, anti-caking agent, germicide, lubricant, ore floating agent, emulsifier, water treatment agent and insecticide.
29.	{[Thorium-(pentamethylcyclopentadienyl)]-tris[(trimethylsilylamino)-1′,2′-ethylideneamino]}	39.43	$C_{25}H_{54}N_4Si_3Th$	726	4.80	Aromatic compound	No activity reported
30.	Methyl6-deoxy-6-isothiocyanato-2,3,4-tri-o-trimethylsilylà- D-galactopyranoside	39.82	$C_{17}H_{37}NO_5Si_3$	451	2.40	Organic compound	No activity reported

[a] Source: Dr. Duke's Phytochemical and Ethnobotanical Databases.

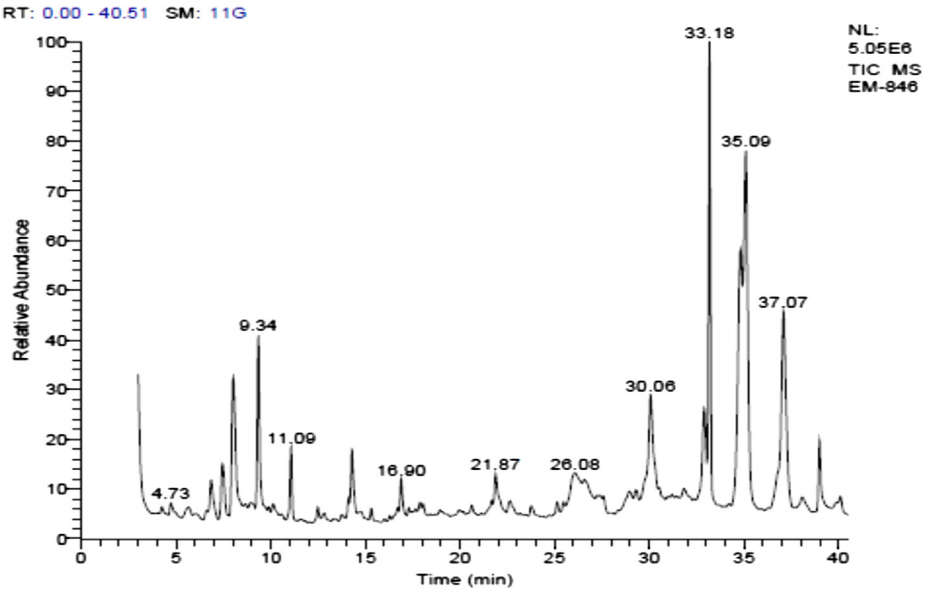

Fig. 1. GC–MS chromatogram of methanolic leaf extract of *Coscinium fenestratum.*

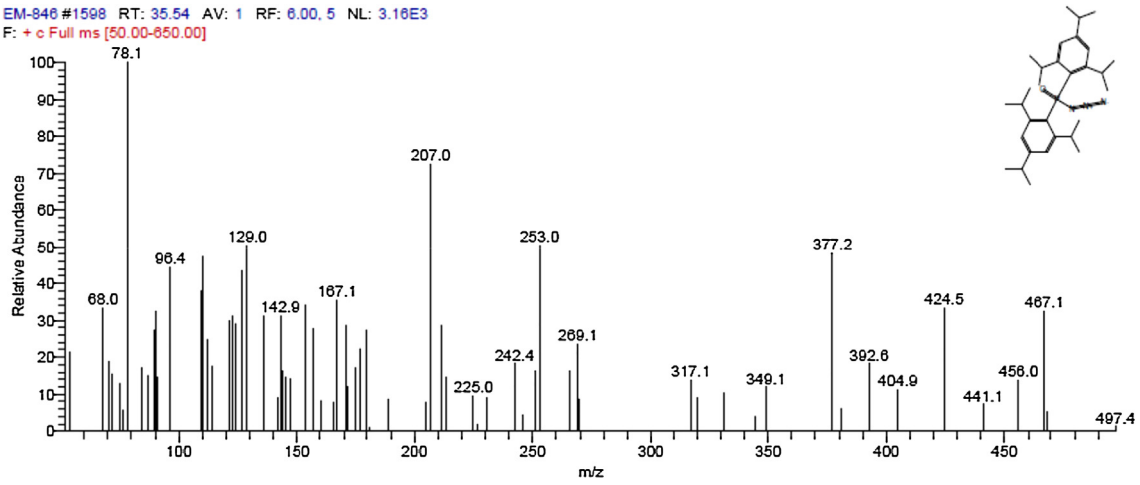

Fig. 2a. Mass spectrum of Bis(2,4,6-triisopropylphenyl) phosphinicazid.

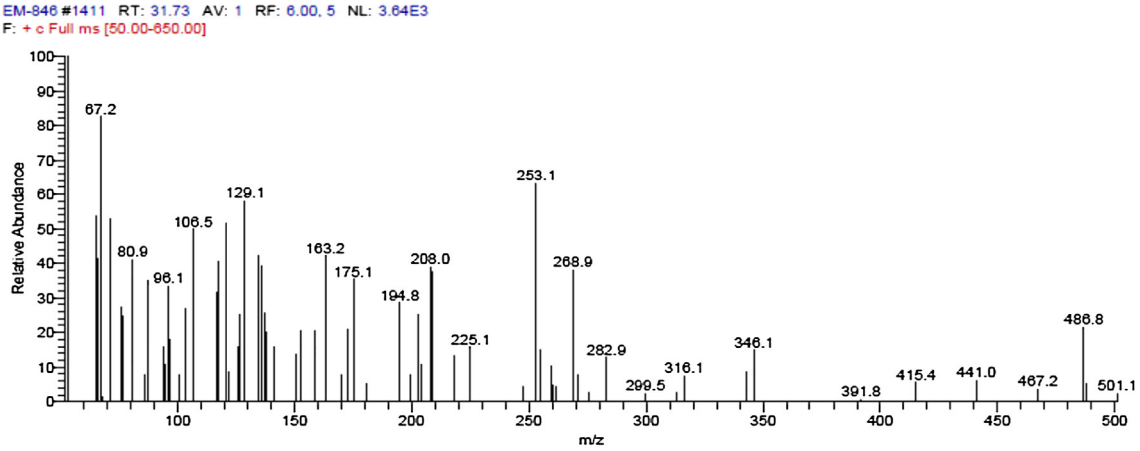

Fig. 2b. Mass spectrum of Methyl2-N-cyclohexylamino-2,3-dideoxy-4,6-O-(phenylmethylene) -3-C-phenylsulfonyl-à,D-glucopyranoside.

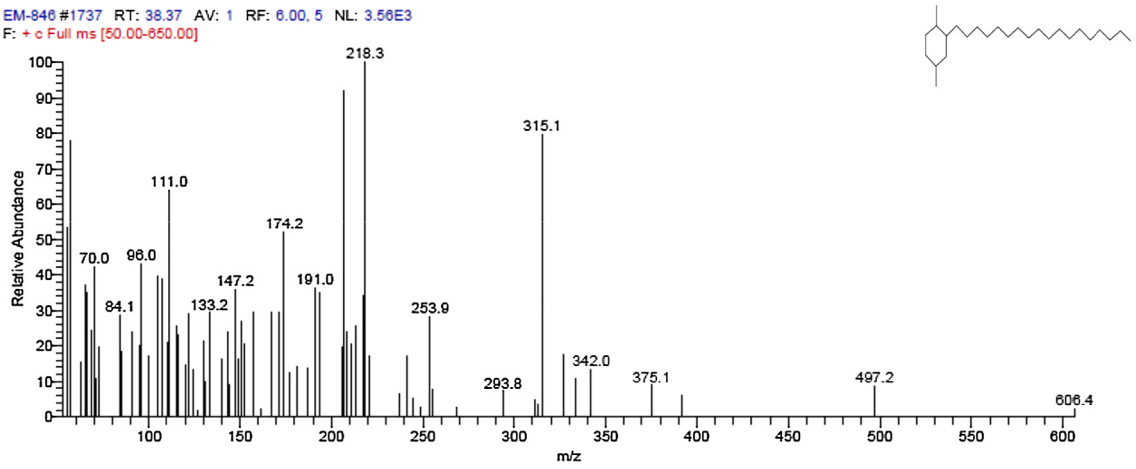

Fig. 2c. Mass spectrum of Cyclohexane, 1,4-dimethyl-2-octadecyl- (CAS).

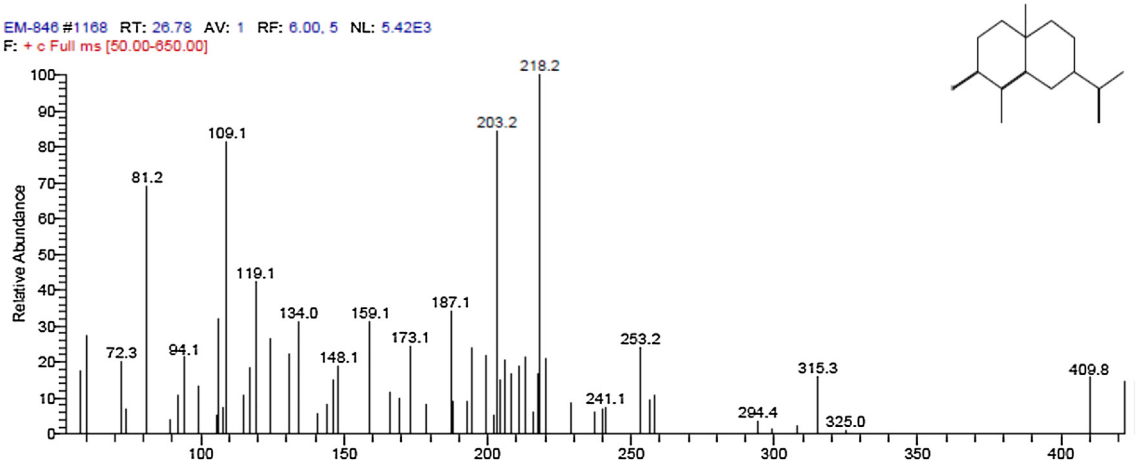

Fig. 2d. Mass spectrum of à-Cyperone.

Table 3
Reducing power activity of MeOHCf compared with certain standard antioxidants.

Sample concentration (μg/ ml)	Leaf extract (absorbance at 700 nm)	Sample concentration (μg/ ml)	Rutin	BHA	Quercitin	BHT
50	0.610 ± 0.03[a]	20	0.238 ± 0.003[a]	0.236 ± 0.016[b]	0.359 ± 0.012[a]	0.224 ± 0.001[a]
100	0.645 ± 0.02[a]	40	0.350 ± 0.013[c]	0.396 ± 0.017[c]	0.632 ± 0.023[b]	0.368 ± 0.009[b]
150	0.723 ± 0.06[b]	60	0.408 ± 0.013[c]	0.496 ± 0.028[d]	0.718 ± 0.019[c]	0.478 ± 0.013[c]
200	0.816 ± 0.04[c]	80	0.476 ± 0.006[b]	0.593 ± 0.008[a]	0.833 ± 0.044[d]	0.517 ± 0.017[d]
250	1.060 ± 0.07[d]	100	0.557 ± 0.014[c]	0.644 ± 0.011[b]	0.973 ± 0.029[e]	0.584 ± 0.012[e]

Values were performed in triplicates and represented as mean ± SD.
Mean values followed by different superscript in a column are significantly different ($p < 0.05$).

Table 4
DPPH· scavenging activity of MeOHCf compared with certain standard antioxidants.

Sample concentration (μg/ mL)	% of inhibition	IC_{50} value (μg/mL)	Standard antioxidants	IC_{50} value (μg/ mL)
50	32.54 ± 0.05[a]	182.48	Rutin	15.75 ± 0.01
100	32.74 ± 0.04[a]		Quercetin	20.72 ± 0.05
150	44.21 ± 0.03[b]		BHA	21.42 ± 0.11
200	50.09 ± 0.07[bc]		BHT	34.74 ± 0.26
250	64.80 ± 0.04[c]			

Values are performed in triplicates and represented as mean ± SD.
Mean values followed by different superscripts in a column are significantly different ($p < 0.05$).

required bio compounds for scavenging activity. Similar trend of this activity was also documented previously in our laboratory (Karthika et al., 2014). MeOHCf exhibited higher $ABTS^{\bullet+}$ scavenging activity. The 2,2′-azinobis (3-ethylbenzothiazoline sulphonate) radical cation ($ABTS^{\bullet+}$) scavenging activity was 2453.7 μmol trolox equivalent/ g extract (Table 4). This high activity could be due to abundance of secondary metabolites in the plant extracts (Rojsanga et al., 2006; Tushar et al., 2008).

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Conflict of interest

The authors report no conflicts of interest in this work.

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