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ORIGINAL ARTICLE

Extraction and identification of bioactive components in *Sida cordata* (Burm.f.) using gas chromatography–mass spectrometry

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Abstract *Sida cordata* (Burm.f.) is a pineal tropical plant in the family Malvaceae that is found throughout India and used to treat various diseases and ailments in many complementary and alternative medicine systems. This study identified the bioactive components present in whole-plant ethanol extracts of *S. cordata* using gas chromatography–mass spectrometry (GC–MS). Based on their retention times (RT) and mass-to-charge ratios (m/z), 29 bioactive compounds were identified: nonanoic acid, vitamin D_3, 3-trifluroacetoxypentadecane, α-D-glucopyranoside, O-α-D-glucopyranosyl-(1.fwdarw.3)-α-D-fructofuranosyl,3,7,11,15-tetramethyl-2-hexadecan-1-ol, octadecanoic acid, ethyl ester, phytol, 9,12-octadecadienoic acid, methyl ester (E,E), 9,12,15-octadecadienoic acid, methyl ester (Z,Z,Z), oleic acid, 1,2-15,16-diepoxyhexadecane, 3-hexadecyloxycarbonyl-5-(2-hydroxyethyl)-4-methylimidazolium ion, methoxyacetic acid, 4-tetradecyl ester, 1,2-benzenedicarboxylic acid, mono (2-ethylhexyl) ester, 1-iodo-2-methylundecane, dodecane, 2,6,10-trimethyl-, 2-piperidinone-N-[4-bromo-n-butyl]-, squalene, octadecane-1-(ethenyloxy)-, Z,Z-2,5-pentadecadien-1-ol, 1-hexadecanol, 2-methyl-, spiro[androst-5ene-17,1′-cyclobutan]-2′-one-3-hydroxy-, (3a,17a)-, diethylene glycol monododecyl ether, vitamin E, cholestan-3-ol, 2-methylene-, (3a,5a)-, 2H-pyran, 2-(7-heptadecynyloxy)tetrahydro-, and *cis*-Z-α-bisabolene epoxide. The presence of various bioactive compounds justifies the use of this plant for treating various ailments by traditional practitioners.

Keywords *Sida cordata* · GC–MS · Extraction · Bioactive compounds

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Introduction

Aspects of the modern lifestyle, such as smoking, overconsumption of alcohol, and fast foods with excessive colorants and chemical preservatives place severe oxidative stress on cells and body systems, leading to the production of free radicals. These free radicals cause oxidative damage to lipids, proteins, and nucleic acids, which leads to diseases such as atherosclerosis, cancer, diabetes, inflammation, and Alzheimer's and other degenerative diseases (Fransen et al. 2012). Many plant secondary metabolites are potential free radical scavengers, including flavonoids, anthocyanins, carotenoids, dietary glutathione, polyphenols, vitamins, and endogenous metabolites. Free radical scavengers are antioxidants that accept electrons from the free radicals produced in vivo or in vitro. Rutin, morin, quercetin (flavonoids), naringenin (flavone), catechin (flavanol), retinol and tocopherol (vitamins), and curcumin (polyphenol) are well-studied plant-derived secondary metabolites that possess anti-cancer, free radical scavenging, anti-ulcer, and antimicrobial activities. Flavanols are related to catechins, quercetin, and kaempferol, and their glycosides are found in beverages such as green and black teas and red wines. Quercetin occurs in onions and apples, while berries contain myricetin and quercetin. These dietary compounds protect against oxidative stress.

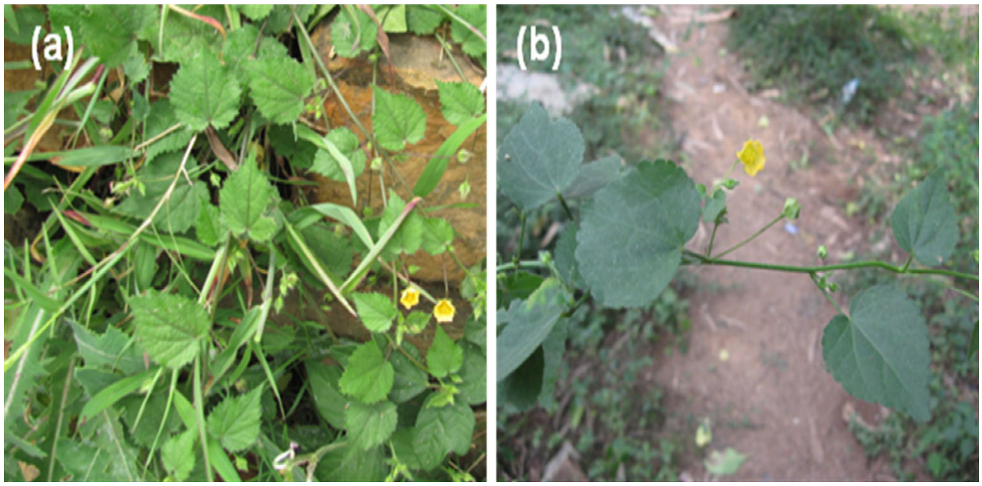


Fig. 1 Photographic Images of *S. cordata* **a** whole plant **b** magnified image of stem with flowering *top*

Table 1 Preliminary phytochemical screening results of ethanolic extract of *Sida cordata*

Phytoconstituents	Test performed	Ether	Ethanolic extract	Water
Alkaloids	Mayer's test	−	++	+
	Wager's test	−	++	+
	Dragendorff test	−	++	−
	Hager's test	−	++	−
Terpenoids	Liebermann test	−	++	+
	Salkowski test	−	++	+
Saponins	Forth test	+	+	+
	Foam test	+	+	+
Carbohydrates	Molisch's test	−	+	++
	Benedict's test	−	+	++
Flavonoids	Alkaline reagent test	−	++	++
	Lead acetate test	−	++	+
Glycosides	Borntrager's test	+	+	+
Anthraquinones	Sulphuric acid test	−	+	−

High concentration (++), Moderate concentration (+), Nil (−)

Many active pharmaceuticals have been derived from plant secondary metabolites, such as vinca alkaloids and Taxol, which effectively treat cancers (Cragg and Newman 2005). Hence, it is important to isolate natural antioxidants from plants. The initial steps are extraction and separation of the active phytochemicals from plants before identifying their active ingredients (Karimi and Jaafar 2011). Methods for identifying such compounds should be simple and repeatable. One of the best methods for identifying these compounds is gas chromatography–mass spectrometry (GC–MS), which can isolate and analyze compounds in a single step using a mass detector and available GC–MS libraries (Gomathi et al. 2015).

Sida cordata (Malvaceae) is a small perennial tropical weed found throughout India, as well as in some other Asian countries. It is commonly known as the long-stalk sida or *kurunthotti* in Tamil. In Indian alternative medicine, the entire plant is used for making medications such as Siddha and Ayurveda. Its roots are used as a diuretic to treat urinary problems and its seeds and oil extract are used as laxatives, aphrodisiacs, and demulcents. *S. cordata* is recommended in cystitis, colic gonorrhea, and piles (Gnanasekaran et al. 2012; Shah et al. 2014). The abortifacient effect of its ethanol extract has also been reported (Shah et al. 2014). It has hepatoprotective effects in vitro (Mistry et al. 2013; Shah et al. 2013). However, no study has identified the active constituents that are responsible for the therapeutic effects of *S. cordata*.

Therefore, we identified the active molecules present in this medicinally valuable plant using simple solvent extraction followed by GC–MS separation.

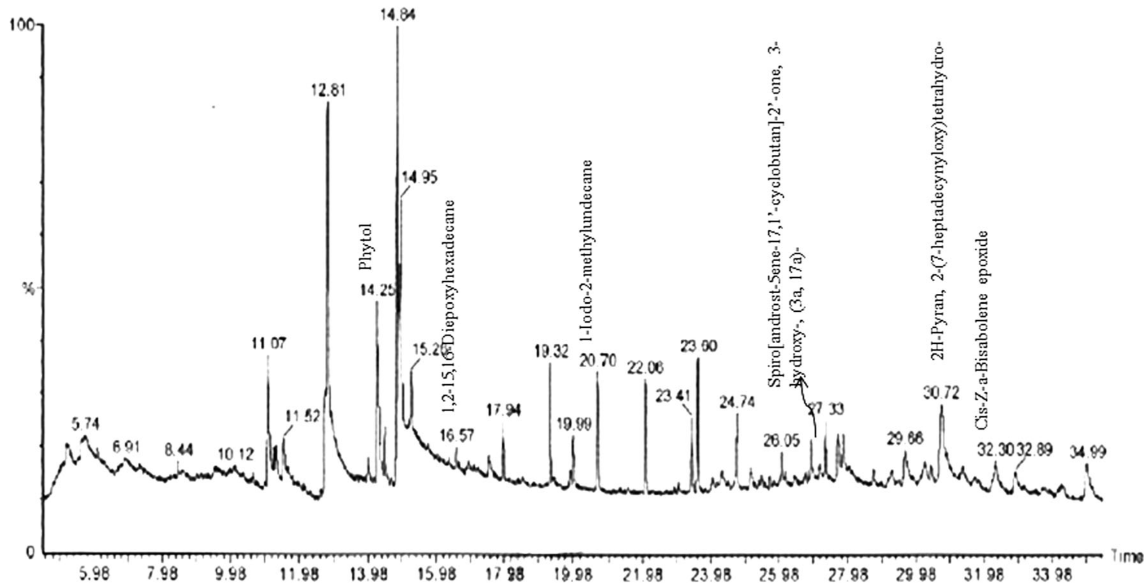


Fig. 2 GC–MS traces of bio-active constituents of *S. cordata* ethanolic extract

Materials and methods

Preparation of extract

Fresh whole *S. cordata* plants were collected from Tirupati, Andhra Pradesh, India. The plant material was identified taxonomically and authenticated by Professor. K. Madhava Chetty, Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, India. A voucher specimen (no. 874) was stored in the department's herbarium (Fig. 1). Approximately 5 kg of *S. cordata* were dried in the shade for 2 weeks, coarsely powdered, and sieved with #40 mesh. The dried material weighed about 1 kg. The powdered plant material was stored in an airtight container at room temperature until further use. Small quantities of the dried powdered material were preliminarily extracted with various solvents of different polarities, including ether, water, and ethanol. Ethanol was found to be the best solvent for extraction. Then, approximately 500 g of the dried powder was extracted with ethanol using hot continuous extraction with soxhlation at 60 °C. The extracts were then reduced to a dried powder using a rotary evaporator and stored at 4 °C until use.

Qualitative phytochemical analysis

Preliminary evaluation of the extracted phytochemicals was performed using the methods of Harborne (1984), Trease and Evans (1989), and Kokate (1994) to test for the presence of steroids, carbohydrates, terpenoids, alkaloids, flavonoids, and saponins.

Gas chromatography–mass spectrometry analysis

The GC–MS analysis was performed using a Perkin-Elmer GC Clarus 500 gas chromatograph system interfaced with a mass spectrometer equipped with an Elite −5 MS column (5% diphenyl/95% dimethyl poly siloxane, 30 × 0.25 mm × 0.25 μm df). For GC–MS detection, an electron ionization system with ionizing energy of 70 eV and helium (99.999%) carrier gas at a constant flow rate of 1 mL/min, with an injection volume of 2 μL (extract dispersed in acetone, ultrasonicated for 15 min and filtered through a 0.22 μm nylon filter before injection), was injected with a split ratio of 10:1. The injector temperature was kept at 250 °C; the ion-source temperature was maintained at 200 °C. The oven temperature was programmed to increase from 110 °C (isothermal for 2 min) to 200 °C at 10 °C/min, and then to 280 °C at 5 °C/min; it was held at this temperature for 9 min. Mass spectra were then taken at 70 eV, with a scan interval of 0.5 s and fragments from 45 to 450 Da. The total GC run time was 36 min. The relative percentage of each component was calculated by comparing its average peak area to the total area using TurboMass software (ver. 5.2).

Compound identification

The isolated peaks of the plant extract were compared with known spectra in the National Institute of Standards and Technology database, which contains 62,000 patterns.

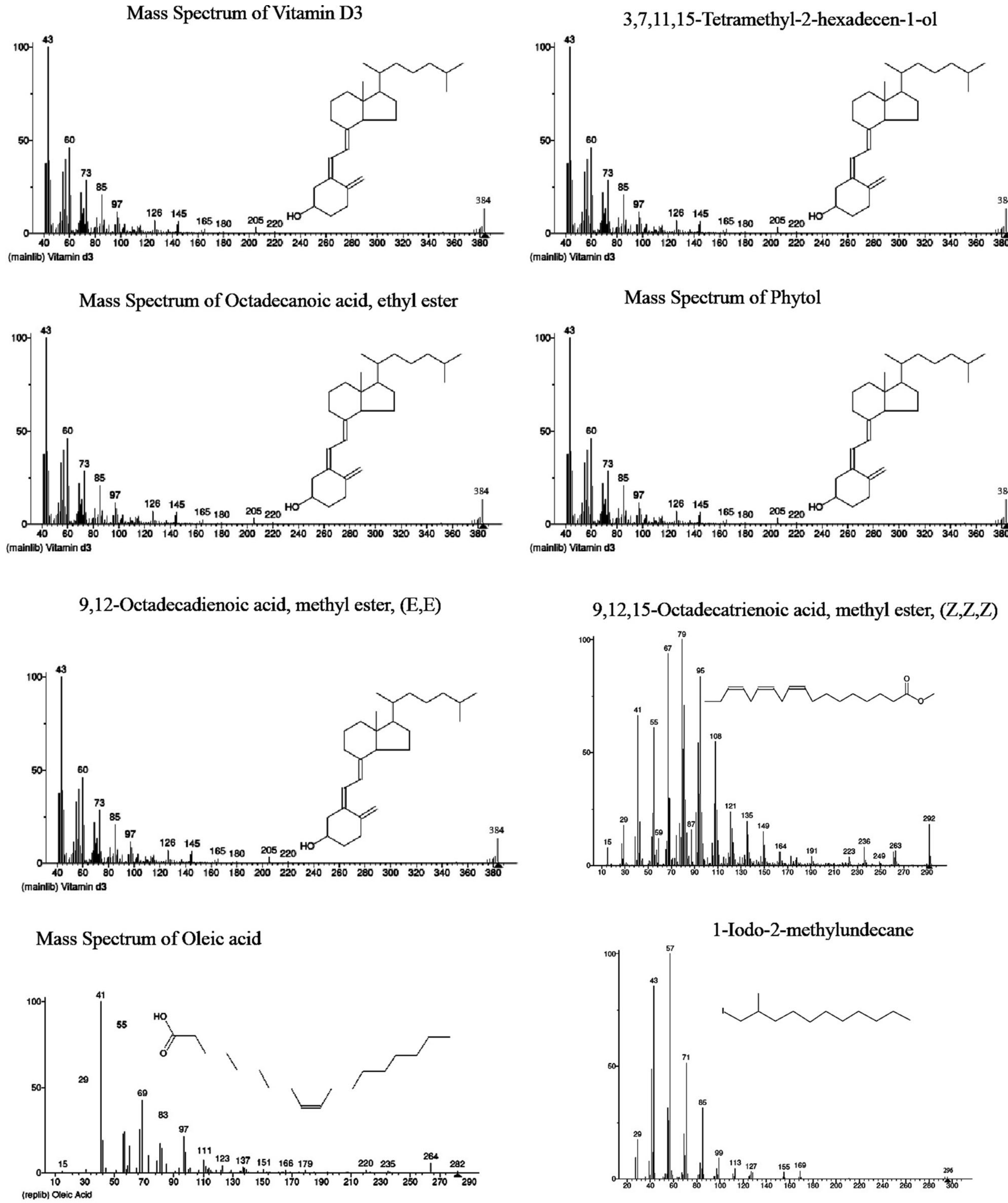
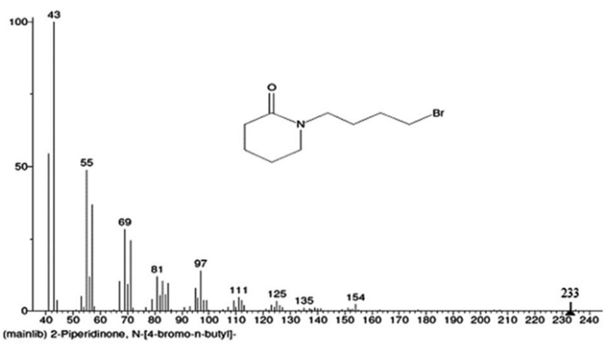
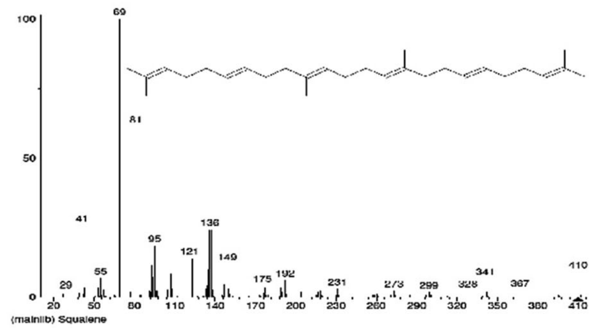


Fig. 3 Mass spectrums of various phyto constituents indentified from the extract

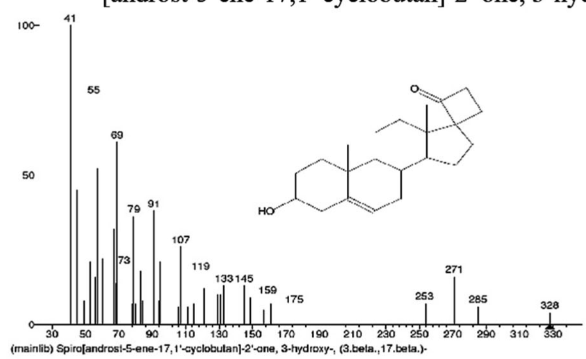
Mass Spectrum of 2-Piperidinone, N-[4-bromo-n-butyl]



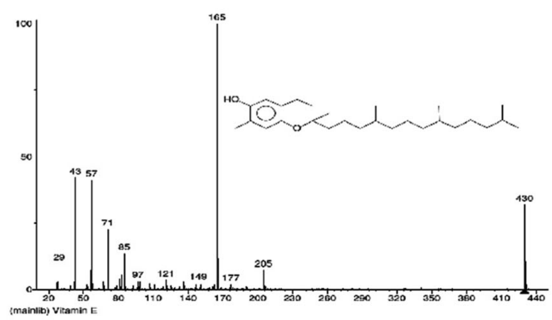
Mass Spectrum of Squalene



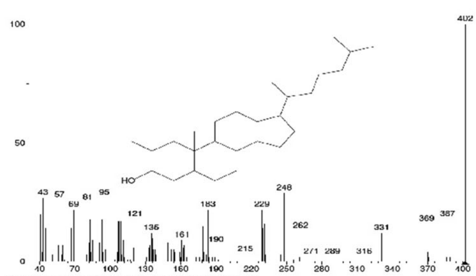
Mass Spectrum of Spiro
[androst-5-ene-17,1'-cyclobutan]-2'-one, 3-hydroxy-,



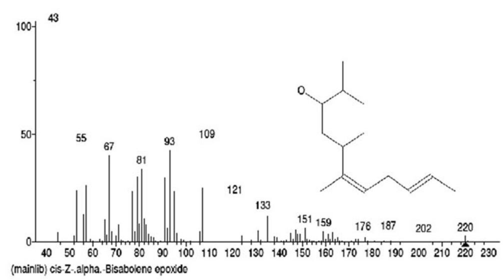
Mass Spectrum of Vitamin E



Mass Spectrum of Cholestan-3-ol, 2-methylene-, (3a,5a)-



Mass Spectrum of cis-Z-à-Bisabolene epoxide



Mass Spectrum of 1- Heptatrioctanol

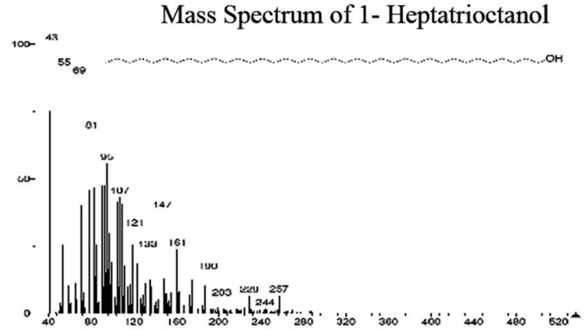


Fig. 3 continued

Table 2 Bio-active components identified in the *S. cordata* whole plant by GC–MS analysis

S. no.	RT	Name of the compound	Mol. formula	Mol. weight	Peak area %
1	5.74	Nonanoic acid	$C_9H_{18}O_2$	158	14.18
2	6.91	Vitamin D_3	$C_{27}H_{44}O$	384	23.53
3	8.44	3-Trifluroacetoxypentadecane	$C_{17}H_{31}F_3O_2$	324	0.64
4	10.12	a-D-Glucopyranoside, O-a-D-glucopyranosyl-(1.fwdarw.3)-a-D-fructofuranosyl	$C_{18}H_{32}O_{16}$	504	5.61
5	11.07	3,7,11,15-Tetramethyl-2-hexadecan-1-ol	$C_{20}H_{40}O$	296	3.74
6	12.81	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312	15.30
7	14.25	Phytol	$C_{20}H_{40}O$	296	3.49
8	14.84	9,12-Octadecadienoic acid, methyl ester, (E,E)-	$C_{19}H_{34}O_2$	294	3.89
9	14.95	9,12,15-Octadecadienoic acid, methyl ester, (Z,Z,Z)-	$C_{19}H_{32}O_2$	292	23.89
10	15.26	Oleic acid	$C_{18}H_{34}O_2$	282	0.85
11	16.57	1,2-15,16-Diepoxyhexadecane	$C_{16}H_{30}O_2$	254	0.23
12	17.94	3-Hexadecyloxy carbonyl-5-(2-hydroxyethyl)-4-methylimidazolium ion	$C_{24}H_{45}N_2O_3$	409	0.75
13	19.32	Methoxy acetic acid, 4-tetradecyl ester	$C_{17}H_{34}O_3$	286	1.55
14	19.99	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	$C_{16}H_{22}O_4$	278	0.77
15	20.70	1-Iodo-2-methylundecane	$C_{12}H_{25}I$	296	1.69
16	22.06	Dodecane, 2,6,10-trimethyl-	$C_{15}H_{32}$	212	1.47
17	23.41	2-Piperidinone, N-[4-bromo-n-butyl]-	$C_9H_{16}BrNO$	233	1.07
18	23.60	Squalene	$C_{30}H_{50}$	410	1.69
19	24.74	Octadecane, 1-(ethenyloxy)-	$C_{20}H_{40}O$	296	1.14
20	25.14	Z,Z-2,5-Pentadecadien-1-ol	$C_{15}H_{28}O$	224	0.46
21	26.05	1-Hexadecanol, 2-methyl-	$C_{17}H_{36}O$	256	0.50
22	26.91	Spiro[androst-5ene-17,1′-cyclobutan]-2′-one, 3-hydroxy-, (3a, 17a)-	$C_{22}H_{32}O_2$	328	0.84
23	27.33	Diethylene glycol monododecyl ether	$C_{16}H_{34}O_3$	274	0.84
24	27.84	Vitamin E	$C_{29}H_{50}O_2$	430	31.45
25	29.66	Cholestan-3-ol, 2-methylene-, (3a,5a)-	$C_{28}H_{48}O$	400	1.31
26	30.72	2H-Pyran, 2-(7-heptadecynyloxy)tetrahydro-	$C_{22}H_{40}O_2$	336	5.03
27	32.30	Cis-Z-a-Bisabolene epoxide	$C_{15}H_{24}O$	220	1.37
28	32.89	9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1-[[(trimethylsilyl)oxy]methyl]ethyl ester, (Z,Z,Z)-	$C_{27}H_{52}O_4Si_2$	496	0.95
29	34.99	1-Heptatriacotanol	$C_{37}H_{76}O$	536	1.80

Results and discussion

Phytochemical profile

Preliminary investigation of the ethanol extract of *S. cordata* showed the presence of steroids, carbohydrates, terpenoids, alkaloids, flavonoids, and saponins (Table 1). Therefore, we selected the ethanol extract for further isolation studies using GC–MS.

GC–MS analysis

Figure 2 shows a full scan gas chromatogram of the ethanol extract of *S. cordata*. It confirmed the presence of various bioactive compounds with different retention times (RT). The peaks of each component were obtained from the mass spectra and are shown in Fig. 3. The compounds identified by their RT, molecular weight, and percentage peak area are illustrated in Table 2, along with their molecular formulas. Table 3 summarizes the nature of the identified compounds and their biological activities, as predicted from Dr. Duke's phytochemical and ethanobotanical databases (U.S. Department of Agriculture, Agricultural Research Service 1992–2016).

Twenty-nine compounds were detected in the ethanol extract of *S. cordata*. Based on the RT and peak area of individual bioactive compounds, the predominant compounds were vitamin E (31.45%), 9,12,15-octadecadienoic acid, methyl ester, (Z,Z,Z) (23.89%), vitamin D_3 (23.53%), octadecanoic acid, ethyl ester (15.30%), α-D-

Table 3 Activity of phytocomponents identified in the ethanolic extract of *S.cordata* whole plant

S. no.	Name of the compound	Nature	Biological activity	References
1	Nonanoic acid	Carboxylic acid	Antimicrobial	Nurettin et al. 2006
2	Vitamin D_3	Steroid	Steroid hormone	How et al. 1994
3	3-Tri fluro acetoxy pentadecane	Acidic compound	Anti-nephrotoxic and antioxidant activities	Haider et al. 2016
4	a-D-Glucopyranoside, O-a-D-glucopyranosyl-(1.fwdarw.3)-a-D-fructofuranosyl	Basic Sugars (Mono and Oligosaccharides)	No activity reported	–
5	3,7,11,15-Tetramethyl-2-hexadecen 1-ol	Terpene alcohol	Antimicrobial, anti-inflammatory	Sudha et al, 2013
6	Octadecanoic acid, ethyl ester	Stearic acid ester	Antioxidant, anti-inflammatory	Dr. Dukes
7	Phytol	Diterpene alcohol	Antinociceptive, Antioxidant, anticancer, anti-inflammatory, antimicrobial, diuretic, chemopreventive properties	Camila et al. 2013
8	9,12-Octadecadienoic acid, methyl ester, (E,E)-	Linolelaidic acid ester	Hepatoprotective, antihistaminic, hypocholesterolemic, antieczemic	Dr. Dukes
9	9,12,15-Octadecadienoic acid, methyl ester, (Z,Z,Z)-	Linolenic acid, methyl ester	Antiinflammatory, Hypocholesterolemic, Cancer preventive, Hepatoprotective, Nematicide, Insectifuge, Antihistaminic, Antieczemic, Antiacne, 5-Alpha Reductase inhibitor, Antiandrogenic, Antiarthritic, Anticoronary, Insectifuge	Rehana and Nagarajan 2013
10	Oleic acid	Steric acid	Anti-inflammatory, anti-androgenic, anti-cancer, preservative and hypocholesterolemic	Sreekumar et al. 2014
11	1,2-15,16-diepoxyhexadecane	Epoxide	Antitumor, anti-inflammatory	Imad et al. 2016
12	3-Hexadecyloxy carbonyl-5-(2-hydroxyethyl)-4-methylimidazolium ion	Imidazole	Antifungal, Antibacterial	Subavathy and Thilaga 2016
13	Methoxyacetic acid, 4-tetradecyl ester	Acidic compound	Anti-microbial	Agnel and Mohan 2014
14	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	Quinoline	Cytotoxic	Krishnan et al. 2014
15	1-Iodo-2-methylundecane	Iodine compound	Estrogen	Achiraman et al. 2010
16	Dodecane, 2,6,10-trimethyl-	Alkane	No activity reported	–
17	2-Piperidinone, N-[4-bromo-n-butyl]-	Alkaloid	Antimicrobial Anti-inflammatory	Dr. Dukes
18	Squalene	Triterpene	Anti-oxidant, Anti-tumor	Ryszard 2009
19	Octadecane, 1-(ethenyloxy)-	Alkane	No activity reported	–
20	Z,Z-2,5-Pentadecadien-1-ol	Unsaturated alcoholic compound	No activity reported	–
21	1-Hexadecanol, 2-methyl-	Alcoholic compound	Anti-microbial	Sarada et al. 2011
22	Spiro[androst-5ene-17,1′-cyclobutan]-2′-one, 3-hydroxy-, (3a, 17a)-	Steroid	Antimicrobial, Anticancer, Anti-inflammatory, Diuretic, Anti-asthmatic, Anti-arthritic	Archana et al. 2014
23	Diethylene glycol monododecyl ether	Ether compound	Surfactant	Bandyopadhyay and Chanda 2003
24	Vitamin E	Tocopherol	Antioxidant	Traber and Atkinson 2007
25	Cholestan-3-ol, 2-methylene-, (3a,5a)-	Steroid	Antimicrobial, anticancer, diuretic, anti-asthma, anti-arthritic	Jegadeeswari et al. 2012
26	2H-Pyran, 2-(7-heptadecynyloxy)tetrahydro-	Flavonoid	Antimicrobial Anti-inflammatory Antioxidant	Amutha and Kottai 2014

Table 3 continued

S. no.	Name of the compound	Nature	Biological activity	References
27	Cis-Z-a-Bisabolene epoxide	Pheromone compound	To increase sex hormone activity	Amutha and Kottai 2014
28	9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1-[[(trimethylsilyl)oxy]methyl]ethyl ester, (Z,Z,Z)-	Silica compound	No activity reported	–
29	1-Heptatriacotanol	Alcoholic compound	Anti-microbial	Kalairasan et al. 2011

glucopyranoside, O-α-D-glucopyranosyl-(1.fwdarw.3)-α-D-fructofuranosyl (5.61%), 2H-pyran, 2-(7-heptadecynyloxy)tetrahydro- (5.03%),9,12-octadecadienoic acid, methyl ester, (E,E) (3.89%), 3,7,11,15-tetramethyl-2-hexadecan-1-ol (3.74%), and phytol (3.49%). Their chemical structures were predicted using the mass spectra based on their fragmentation, which generates peaks with different mass-to-charge ratios (m/z).

The use of medicinal plants in the treatment of various human ailments depends on their phytochemical constituents. This study revealed that the ethanol extract of *S. cordata* contained 29 compounds. Our preliminary investigation of the presence of various active constituents in water, ethanol, ether, and ethyl acetate extracts indicated that ethanol extracted the most phytochemicals from the plant. Hence, we used only the ethanol extract for the GC–MS study. Of the isolated compounds, nonanoic acid (Nurettin et al. 2006), 3,7,11,15-tetramethyl-2-hexadecen 1-ol (Sudha et al. 2013), methoxyacetic acid, 4-tetradecyl ester (Agnel and Mohan 2014), 2-piperidinone, N-[4-bromo-n-butyl]-, (Dr. Duke's), 1-hexadecanol, 2-methyl-(Sarada et al. 2011), spiro[androst-5ene-17,1′-cyclobutan]-2′-one, 3-hydroxy-, (3a, 17a)-, cholestan-3-ol, 2-methylene-, (3a,5a)-, 2H-pyran, 2-(7-heptadecynyloxy)tetrahydro- (Archana et al. 2014), and 1-heptatriacotanol (Kalairasan et al. 2011) possess antimicrobial activity. The antioxidants included 3-trifluroacetoxypentadecane, octadecanoic acid, ethyl ester, squalene, vitamin E, 2-(7-heptadecynyloxy) tetrahydro-, and 2H-pyran (Rao et al. 1998; Traber and Atkinson 2007; Ryszard 2009; Amutha and Kottai 2014; Haider et al. 2016). *cis*-Z-α-bisabolene epoxide is a pheromone that increases sex hormone activity (Amutha and Kottai 2014), while 1-iodo-2-methylundecane acts as an estrogen (Achiraman et al. 2010). Eight compounds had cytotoxic activity (Camila et al. 2013): 9,12,15-octadecadienoic acid, methyl ester, (Z,Z,Z)- (Rehana and Nagarajan 2013), oleic acid (Sreekumar et al. 2014), 1,2-15,16-diepoxyhexadecane (Imad et al. 2016), 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester (Krishnan et al. 2014), squalene (Ryszard 2009, Kala et al. 2011), spiro[androst-5ene-17,1′-cyclobutan]-2′-one, 3-hydroxy-, (3a, 17a)- (Archana et al. 2014), and cholestan-3-ol, 2-methylene-, (3a,5a)- (Jegadeeswari et al. 2012). The phytols promoting reactive oxygen species constitute a promising novel class of pharmaceuticals for the treatment of rheumatic arthritis and possibly other chronic inflammatory diseases (Ogunlesi et al. 2009). No activities have yet been reported for α-D-glucopyranoside, O-α-D-glucopyranosyl-(1.fwdarw-3)-α-D-fructofuranosyl, a basic sugar moiety, octadecane, 1-(ethenyloxy)-, Z,Z-2,5-pentadecadien-1-ol, an ethanol compound, or 9,12,15-octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1-[[(trimethylsilyl)oxy]methyl]ethyl ester (Z,Z,Z).

Several active compounds are unique to *S. cordata*, including [androst-5ene-17,1′-cyclobutan]-2′one, *cis*-Z-α-bisabolene, 1-iodo-2-methylundecane cyclobutan]-2′-one, 3-hydroxy-, and (3a, 17a)-1,2-15,16-diepoxyhexadecane and phytol. Of these [androst-5ene-17,1′-cyclobutan]-2′-one, *cis*-Z-α-bisabolene and 1-iodo-2-methylundecane cyclobutan]-2′-have reported aphrodisiac and abortifacient activities, respectively (Shah et al. 2013); 3-hydroxy-, (3a, 17a)-1,2-15,16-diepoxyhexadecane may be responsible for the anti-inflammatory, antioxidant, and anti-cancer activity reported by Shah et al. (2014). Another phytosterol, vitamin E, may be responsible for the antioxidant activity.

This investigation revealed that *S. cordata* is a potential source of various bioactive compounds, such as esters, alcohols (Sarada et al. 2011), steroids (Archana et al. 2014; How et al. 1994; Kalpanadevi et al. 2012; Bandyopadhyay and Chanda 2003), alkaloids (Dr. Duke's), terpenes (Sudha et al. 2013), and sugars, which justifies the use of this species in traditional medicine. Further studies need to examine molecules that are present at high concentrations and have potential biological activity. In the future, we plan to isolate compounds from different parts of *S. cordata* and evaluate their pharmacological activities.

Conclusion

Twenty-nine compounds were identified from the ethanol extract of whole *S. cordata* plants using GC–MS analysis. The presence of various bioactive compounds justifies the use of the whole plant for treating various ailments by practitioners of traditional Indian medicine. Some of the bioactive secondary metabolites identified may become commercially important phytopharmaceuticals. However, further studies are needed to ascertain their biological and pharmacological activity.

Author contributions Dr. Mohan Kumar collected the plants, performed the extraction and data collection, and wrote the initial manuscript. Dr. Mani Ganesh contributed to the data interpretation and discussion and edited the final article.

Compliance with ethical standards

Conflict of interest The authors have no conflicts of interest to declare.

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