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Isolation and characterization of bioactive compounds from *Euphorbia cotinifolia*

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Abstract

Background: Green plants are found to be an effective reservoir for bioactive molecules and can provide appreciable sources of antimicrobial agents. Antibacterial activity of solvent extracts of *Euphorbia cotinifolia* leaves was tested by agar cup diffusion and broth microdilution methods against some common human pathogenic bacteria viz., *Bacillus cereus*, *Klebsiella pneumonia*, *Enterobacter aerogenes*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Salmonella typhi*. The methanol extract of *Euphorbia cotinifolia* was subjected to a silica gel column, leading to the isolation of a bioactive compound **1**. The structure of compounds was elucidated by spectroscopic techniques and assessed for their antibacterial activity against several human pathogenic bacteria.

Results: The inhibition zone ranged against some common human pathogenic bacteria was 15.25–19.50 mm, 13.50–19.25 mm, 12–18.50 mm, 15–20 mm, and 13–19 mm for ECMF1, ECMF2, ECMF3, compounds **1**, respectively. The MIC was found to be in the range 91–729 μg/ml for the fractions. The inhibition range was recorded between 12–19 and 10–14 mm for methanol and ethyl acetate extracts, respectively. *K. pneumoniae*, *E. aerogenes*, and *B. subtilis* were highly susceptible to methanol extract with the maximum inhibition zone of 19 mm. The MIC of the compound **1** against human pathogens was 78–833 μg/ml.

Conclusion: The present study results suggest that tested plant extracts have moderate to potent antibacterial activity due to the occurrence of phenols and flavonoids in the extracts. The defensive property of natural antibacterials is mainly due to the presence of these major groups, vitamins, phenols, flavonoids, and carotenoids. In the present study, biologically active diterpene was isolated and the structures of the new diterpenoids isolated from *E. cotinifolia* were closely related to an ingenol ester.

Keywords: *Euphorbia cotinifolia*, Isolation, Antibacterial activity, MIC

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Background

Natural products from plants serve as a vast source of compounds with amazing chemical and functional diversity and make significant contributions to drug development programs. A great deal of research has been throughout the world to isolate the secondary metabolites from natural resources [1]. During recent years, extensive attention has been directed towards the exploitation of plant products for the control of different microbial infestations [2]. Since ancient times, man has utilized plants to treat many common infectious diseases and some of these traditional practiced medicines are still used as part of the habitual treatment of various maladies. Despite the possibility of different methods for the discovery of therapeutics, natural products still remain as one of the best reservoirs of new compounds. The use of plant extracts and phytochemicals, both with known and unknown antimicrobial properties, is of great importance to therapeutic treatments. Many medicinal plants are considered to be rich in antimicrobial crude drugs and also as a source for novel compounds with biological activity, with possibly many new modes of action [3].

Euphorbiaceae family plants are well known for the chemical diversity of compounds especially their isoprenoid constituents, and diterpenoids in the majority of the genus having many different skeletons such as tiglianes, jatrophanes, ingenanes, lathyranes, myrstinanes, sesquiterpenoids, flavonoids, and steroids were also obtained. In addition, the isolated compounds from the genus *Euphorbia* extracts have exhibited different biological activities [4]. There are several reports of antibacterial activity of several euphorbia species. A study of phytochemical and antibacterial activity against human pathogens of ethanolic extracts of some Euphorbiaceae members such as *Euphorbia milii*, *Euphorbia hirta*, *Euphoria pulcherrima*, *Euphorbia tithymaloides*, and *Euphorbia prostrata* reported maximum activity against all the bacterial strains where *Euphorbia milii* showed a zone of inhibition of 10 mm [5]. The study of antibacterial properties of flavonoids of leaves from different cacti (*Euphorbia caducifolia*) against some important bacteria (G+ve or G−ve) and reported that they possess strong antibacterial activity against test pathogenic microbes and revealed 7.83 ± 0.21 mm zone of inhibition for free flavonoid [6]. The petroleum ether and methanolic and aqueous extracts of leaves of *Euphorbia hirta* recorded antimicrobial activity against *B. subtilis*, *E. coli*, *S. aureus*, and *S. cerevisiae*, and all the extracts revealed moderate to significant activity in contrast to standard. The phytochemical analysis of petroleum ether and methanolic and aqueous extracts revealed the presence of tannins, related polyphenols, terpenes, anthocyanins, alcohols, steroid-like ß-sitosterol, and ß-amyrin [7].The above studies reported the potential of Euphorbiaceae plants. In the present research, *Euphorbia cotinifolia* Linn. (Caribbean copper plant) was considered for the study. The common name for the species includes smoke tree spurge, Caribbean copper plant, and tropical smoke bush. *E. cotinifolia* is a tropical shrub or small tree with thin leaves deciduous that is prominent for its attractive burgundy-red foliage. The *Euphorbia* genus belongs to the family Euphorbiaceae that comprises at least 2100 species and is one of the most diverse genera in the plant kingdom [8]. *Euphorbia cotinifolia* is a deciduous tropical shrub possessing many medicinal applications. The leaves of the plant had been employed as poison for catching fish by Southern American Indians. The latex is strongly purgative and the leaves have molluscicidal and antiviral properties [9]. Hirota et al. have worked on the extract of *E. cotinifolia* leaves resulting in isolation and characterization of some ingenol-esters such as 3-O-propionyl-20-O-(S)-(2′-methyl)butyryl-ingenol, 20-*O*-isobutyryl-ingenol, 3-Opropionyl-20-O-isobutyryl-ingenol, and 3,20-O-di-isobutyryl-ingenol which are piscicidal constituents [10]. Recent research [11] on the chemical constituent of *E. cotinifolia* showed the presence of metalloprotease in the latex of *E. cotinifolia*.

This article describes the isolation, characterization, and determination of the structure of the new isolated compound from *E. cotinifolia*. The newly isolated compound was tested for antimicrobial activity against bacterial pathogens.

Methods

Plant material

Healthy and fresh leaves of *Euphorbia cotinifolia* were collected from local areas of Mysore and was used for the preparation of different solvent extracts. A voucher specimen of the plant material has been deposited in the Herbarium, Department Botany of University, and the voucher/specimen number is MGBH01.

Extraction

The test plants were thoroughly washed, shade dried, and powdered by using a warring blender. soxhlet extraction apparatus was used for extraction. One hundred grams of powdered leaf material was placed in a porous thimble of the apparatus in the upper chamber. Two hundred grams of extracting solvent was added to the lower boiling flask. The flask was heated by using a heating mantle controlled by a thermostat. Different solvents based on polarity from low to high in the following order of petroleum ether, chloroform, ethyl acetate, and methanol were filled in the round bottom flask, and the temperature was set based on the boiling point of the solvents. The solvent was heated to reflux and extracted. The material in the thimble was extracted with the different solvents successively till colorless extract was collected on the top of the extractor. The solvent extract collected after was concentrated separately under reduced pressure. After complete evaporation of the solvent from the extract, all solvent extracts were weighed

and preserved in brown airtight bottle at 5 °C until further use.

Phytochemical analysis

Phytochemical analysis of all the solvent extracts was performed for the detection of active secondary metabolites or different constituents such as tannins, alkaloids, flavonoids, terpenoids, steroids, carbohydrates, proteins, and saponins. The dried extracts extracted by soxhlet apparatus were reconstituted in methanol, and each extract was subjected to standard phytochemical analysis according to the procedure described by Harborne [12].

Human pathogenic bacteria

Authentic and pure sample cultures of human pathogenic bacteria viz., *Escherichia coli* (*E. coli*) (MTCC 7410), *Enterobacter aerogens* (*E. aerogens*) (MTCC 7325), *Bacillus cereus* (*B. cereus*) (MTCC 1272), *Bacillus subtilis* (*B. subtilis*) (MTCC 121), *Salmonella typhi* (*S. typhi*) (MTCC 733), *Klebsiella pneumoniae* (*K. pneumoniae*) (MTCC 7407), and *Staphylococcus aureus* (*S. aureus*) (MTCC 7443) were used as test bacteria and obtained from Microbial Type Culture Collection (MTCC), Chandigarh, India. All the above bacterial samples were further sub-cultured on nutrient agar (NA) medium and frequently cultured. These bacterial cultures were used as test pathogens for the assay.

Antibacterial activity

Antibacterial activity of the fractions and compounds obtained was assayed by agar cup diffusion method [13]. On NA medium, 7 mm cork borer was used to make cups. Fifty microliters of 24-h bacterial culture containing 106 CFU/ml of bacteria was transferred and spread on the solidified media with a sterile swab which is moistened with the bacterial suspension. The fractions and compounds to be tested were reconstituted in methanol and prepared at a concentration of 100 mg/ml. One hundred microliters of the fractions and compounds was placed in an individual cup and methanol of 100 µl was placed in the central cup, which was considered as the negative control. All the plates were incubated at 37 °C for 24 h and inhibition zone if any around the cups was measured in millimeters. For each treatment, a set of three replicates were maintained and all assays were repeated twice.

Minimum inhibitory concentration (MIC)

MIC was determined in 96-well sterile flat-bottom microtiter plates based on microdilution assay which is an automated turbidometric and colorimetric method as described by Kuntal Das [14]. Test bacterial inoculum was prepared from 24-h cultured bacteria and a suspension was made in sterile/saline water and adjusted to 0.5 McFarland standard solution turbidity.

E. cotinifolia methanol fractions and compounds were diluted to a concentration of 100 mg/ml which was used as a stock solution. The 96-well microtiter plates were prepared by transferring 200 µl of broth and 100 µl of the fractions/compound to the first well. A twofold serial dilution was made in the row up to 12-well and final concentrations from well 1 to 12 were 5–0.019 mg/ml. A 10-µl inoculum suspension of each bacterial strain was added to each well. The wells containing a solvent and nutrient broth with inoculum served as the negative control. The plates were incubated at 37 °C for 24 h, and the absorbance was measured at 620 nm using microplate reader (LT4000, LABTECH Instruments, UK). The minimum concentration that inhibited visible growth of the test bacteria was considered as the MIC based on the readings.

The minimum inhibitory concentration was also detected by adding TTC (10 µl/well) (2, 3, 5-triphenyl tetrazolium chloride) dissolved in water (TTC 2 mg/ml) and incubated under favorable conditions for 30 min [15]. Presence of viable organisms in the wells changed the dye to pink color. The minimum concentration at which there was a color change was taken as the MIC value. All MIC tests were repeated in triplicates.

Isolation and characterization

All the chemicals and solvents used in the present study were of Analytical Reagent grade. Solvents were used as supplied by commercial sources without any further purification. Elemental analysis was carried out on an Elemental Vario EL elemental analyzer. Column chromatographic separation was performed using Merck 7734 silica gel (60–120 mesh), and TLC experiment was carried out with pre-coated Merck silica gel 60 PF254 aluminum sheets; the spots were visualized under UV light. IR spectra were recorded on a JASCO FTIR-8400 spectrophotometer using Nujol mulls. The ^{1}H NMR spectra were recorded on a Varian AC 400 spectrometer instrument in $CDCl_3$ using TMS as the internal standard. Low-resolution mass spectra were obtained on a Varian 1200 L model mass spectrometer (solvent: CH_3OH). Melting points were determined with a Buchi 530 melting point apparatus in open capillaries and are uncorrected.

The methanol extract (4.1 g) of *E. cotinfolia* was applied to silica gel column, eluting with gradient solvent system of $CHCl_3$–CH_3OH to give three fractions, *E. cotinifolia* methanol fraction [ECMF1 (80:20), ECMF2 (50:50), and ECMF3 (20:80)]. The structures of the compounds obtained from methanol extract (ECMF1 and ECMF2) were elucidated by spectroscopic techniques.

Results

Phytochemical analysis

The phytochemical analysis results of *E. cotinifolia* revealed the presence of tannins, steroids, flavonoids, terpenoids,

Table 1 Phytochemical analysis of different solvent extracts of *E. cotinifolia*

Phytochemical compounds	Extracts			
	Petroleum ether	Chloroform	Ethyl acetate	Methanol
Alkaloids	–	–	–	–
Flavonoids	–	–	+	+
Terpenoids	–	–	+	+
Tannins	–	–	+	+
Steroids	+	+	+	+
Glycosides	+	+	+	+
Carbohydrates	+	+	–	–
Proteins	–	–	–	–
Saponins	–	–	–	–

+ = Present. – = Absent

and glycosides in methanol and ethyl acetate extracts. Carbohydrates, steroids, and glycosides were present in petroleum ether and chloroform extracts. The phytochemical analysis of *E. cotinifolia* was studied and the results of these are given in Table 1.

Antibacterial activity

Antibacterial activity results of aqueous and different solvent extracts of *E. cotinifolia* against the test bacteria are presented in Table 2. Among the solvent extracts, methanol and ethyl acetate showed significant activity while negligible activity was found with chloroform extract. The inhibition range was recorded between 12–19 and 10–14 mm for methanol and ethyl acetate extracts, respectively. *K. pneumoniae*, *E. aerogenes*, and *B. subtilis* were highly susceptible to methanol extract with the maximum inhibition zone of 19 mm. Ethyl acetate extract showed a uniform inhibition zone in the range of 10–14 mm against all the tested bacteria. Petroleum ether extract showed minimum activity. The MIC for susceptible test bacteria ranged from 0.312–1.25 mg/ml for methanol and ethyl acetate extracts. The minimum MIC concentration was 0.312 mg/ml recorded for *B. subtilis* for both the extracts (Table 3)

Three fractions were obtained from methanol extract of *E. cotinifolia* and showed activity against all the tested human pathogenic bacteria. The inhibition zone measured for the new compounds was good but slightly lesser than that of its crude extract. The inhibition zone range against human pathogenic bacteria was 16.00–19.50 mm, 13.50–22.25 mm, 14.25–18.50 mm, and 15.0 mm for ECMF1, ECMF2, ECMF3, and compound **1**, respectively (Table 4). The MIC of the compound **1** against human pathogens was 78–833 μg/ml (Table 5).

Characterization of active compounds of *E. cotinifolia*

The methanol extract was subjected to a silica gel column, leading to the isolation of a new compound **1**. Compound **1** was obtained as a pale yellow gum from $CHCl_3$–CH_3OH (80:20) elute. The results obtained from the analytical and different spectral studies are given below: Compound **1**. Anal. calc. for ($C_{28}H_{40}O_6$): C 71.16; H 8.53. found: C 71.13; H 8.51. IR (nujol, cm^{-1}): 3364, 1715, 1670, 1638. ^1H NMR (300 MHz, $CDCl_3$) δ: 0.80 (m, 2H), 0.90 (t, 3H), 1.28 (s, 6H), 1.50–1.80 (bm, 12H),

Table 2 Antibacterial activity of different extracts of *E. cotinifolia* against some human pathogenic bacteria (in mm)

Bacteria	Petroleum ether	Chloroform	Ethyl acetate	Methanol	Streptomycin
Bacillus cereus	–	8.75 ± 0.47^{b}	11.25 ± 0.47^{ab}	12.25 ± 0.50^{c}	21.0 ± 0.40^{bc}
Bacillus subtilis	8.25 ± 0.2^{a}	8.50 ± 0.28^{b}	12.75 ± 0.62^{a}	18.25 ± 0.62^{bc}	20.75 ± 0.47^{bc}
Escherichia.coli	–	8.50 ± 0.28^{b}	10.75 ± 0.25^{b}	13.5 ± 0.64^{a}	23.75 ± 0.47^{a}
Enterobacter aerogenes	–	10.25 ± 0.2^{a}	14.0 ± 0.57^{a}	19.25 ± 0.62^{a}	21.25 ± 0.62^{bc}
Klebsiella pneumoniae	8.0 ± 0.40^{a}	8.75 ± 0.25^{b}	12.75 ± 0.25^{a}	19.0 ± 0.40^{a}	19.75 ± 0.62^{c}
Salmonella typhi	–	8.25 ± 0.25^{b}	11.5 ± 0.28^{ab}	16.0 ± 0.40^{a}	22.5 ± 0.28^{ab}
Staphylococcus aureus	–	0.00 ± 0	10.75 ± 0.25^{b}	15.5 ± 0.28^{ab}	19.25 ± 0.47^{c}

Values are means of four independent replicates. Figures followed by different letters in columns differ significantly when subjected to Tukey ($P < 0.05$). – means no activity

Table 3 MIC of methanol and ethyl acetate extract (mg/mL) of *E. cotinifolia* against some human pathogenic bacteria

Bacteria	*Bacillus cereus*	*Bacillus subtilis*	*Escherichia coli*	*Enterobacter aerogenes*	*Klebsiella pneumoniae*	*Salmonella typhi*	*Staphylococcus aureus*
MIC of methanol	1.25	0.312	1.25	0.625	0.625	0.312	0.312
MIC of ethyl acetate	1.25	0.312	0.625	0.312	0.625	0.625	0.625

1.83 (s, 3H), 2.52 (m, 3H), 3.10 (s, 2H), 3.35 (bs, 1H), 3.68 (s, 3H), 3.86 (d, 1H), 4.73, (d, 1H), 5.40 (s, 1H), 5.80 (d, 1H). ^{13}C NMR (300 MHz, $CDCl_3$) δ: 14, 21, 23, 24, 25, 26, 27, 32, 35, 36, 38, 39, 40, 44, 45, 46, 50, 63, 73, 75, 125, 130, 139, 146, 159, 164, 190. MS, m/z: 472 (M^+).

Based on the above data, the name of the compound **1** is given as *5-hydroxy-4-(2-methoxy-2-oxoethyl)-1,1,7-trimethyl-11-oxo-1a,2,5,5a,6,9,10,10a-octahydro-1H-2,8a-methanocyclopenta [a]cyclopropa[e]*[10]*annulen-6-yl heptanoate* (Fig. 1; Tables 6 and 7).

With the study of the above analytical and spectral data, the following structures are proposed for the newly isolated compound **1** (Fig. 2).

Discussion

Plants are the reservoir of potent biochemicals which are obtained from different parts of the plant material. Herbal remedies from traditional folk medicine are the largely explored field for the development of new active drugs for chemotherapy, by which we can overcome the growing problems of drug resistance and can avoid the toxicity of the currently available antibiotics. The increasing importance in the possible application of the secondary metabolites for human and plant disease management has directed investigation towards the search of new sources of biologically active natural products.

The genus *Euphorbia* belonging to the family Euphorbiaceae is largest comprising about 2000 known species. Several researchers have shown the antibacterial and antioxidant activity of different *Euphorbia* sp. and the reports support the usage of these plants for the treatment of various diseases in traditional medicine [16–18]. The antibacterial activity of ethanol, chloroform, and hexane of extract of leaves of *Euphorbia hirta* extract was studied against some test bacteria and fungi such as *Streptococcus mutans*, *Clostridium absonum*, and *Escherichia coli* by disc diffusion method. The ethanolic leaf extract of *Euphorbia hirta* recorded a maximum zone of inhibition against in *Clostridium* (32 mm) [7, 19]. Phytochemical studies of *Euphorbia milii* showed the presence of cardiac glycosides, steroids/phytosterols, anthocyanin, terpenoids, flavonoids, and tannins, and the hexane extracts in the concentration of 5 μg/ml have shown considerable inhibition zone against *Bacillus subtilis*, *Bacillus megaterium*, *Staphylococcus aureus*, *Entrococci*, *Escherichia coli*, *Proteus vulgaris*, and *Pusodomonas aeruginosa* [20, 21].

E. cotinifolia, the test plant of this study, has shown molluscidal activity [22] and moderate antiviral and cytotoxic activity [23]. Rojas and co-workers [24] have evaluated antibacterial activity of *E. cotinifolia*, where the dried leaves were extracted with isopropyl alcohol and fractionated by column chromatography. Antibacterial activity against *S. aureus*, *Enterococcus faecalis*, *E. coli*, *K. pneumoniae*, and *P. eruginosa* was not reported by any of the fractions eluted from the column separation of *E. cotinifolia* extract. In the present investigation, soxhlet-based extraction successively from low polar to high polar solvents have been reported by the authors. These extracts have been subjected to antibacterial activity against *E. coli*, *K. pneumonia*, *B. subtilis*, *B. cereus*, *S. typhi*, *E. aerogenes*, and *S. aureus*. The toxicity of tannins on microorganisms functions either by direct action on the microbial membrane or by metal ion depletion [25].

Table 4 Antibacterial activity of methanol fractions and compound **1** of *E. cotinifolia* against human pathogenic bacteria (in mm)

Bacteria	Solvent control	ECMF1	ECMF2	ECMF3	Compound 1	Streptomycin	Gentamicin
B. cerus	0.00	16.00 ± 0.00	18.50 ± 0.50	17.50 ± 0.50	15.00 ± 0.00	21.00 ± 0.40	24.30 ± 0.33
B. subtilis	0.00	18.75 ± 1.15	19.25 ± 0.57	18.50 ± 0.57	18.67 ± 0.50	20.75 ± 0.47	29.30 ± 0.88
E. coil	0.00	16.00 ± 0.00	13.50 ± 0.57	14.25 ± 0.57	16.50 ± 0.00	23.75 ± 0.47	22.00 ± 0.57
E. aerogens	0.00	19.50 ± 0.50	22.00 ± 0.57	17.50 ± 0.57	20.00 ± 0.50	21.25 ± 0.62	23.60 ± 0.88
K. pneumonia	0.00	18.00 ± 1.00	17.75 ± 1.15	18.00 ± 0.57	19.50 ± 1.00	19.75 ± 0.62	22.60 ± 0.33
S. typhi	0.00	15.25 ± 0.57	14.50 ± 0.57	14.00 ± 0.00	17.00 ± 1.00	22.50 ± 0.28	23.60 ± 0.66
S. aureus	0.00	17.00 ± 0.00	14.25 ± 0.57	13.50 ± 1.50	17.50 ± 0.00	19.25 ± 0.47	30.30 ± 0.33

Values are mean of three independent replicates. ± Standard deviation

Table 5 MIC of methanol fractions and compound **1** of *E. cotinifolia* against human pathogenic bacteria (in μg/ml)

Bacteria	ECMF1	ECMF2	Compound 1
B. cereus	529	833	625
B. subtilis	208	156	169
E. coil	520	625	729
E. aerogens	78	169	110
K. pneumonia	156	140	156
S. typhi	625	416	833
S. aureus	208	315	416

Values are mean of three independent replicates. ± Standard deviation

Preliminary phytochemical analysis of methanol and ethyl acetate extracts of *E. cotinifolia* revealed the presence of tannins, terpenoids, flavonoids, and steroids. The structure of compounds of *E. cotinifolia* was elucidated by spectroscopic methods (IR, ^{1}H NMR, ^{13}C NMR, and MS) and compared with the earlier literature [13]. The IR spectrum of **1** displayed absorption bands corresponding to hydroxy (3364 cm^{-1}) and carbonyl (1715, 1670, and 1638 cm^{-1}) functionalities. The ^{1}H NMR spectra of compound **1** revealed that the presence of hydroxyl and methyl ester groups, which displayed signals at 3.35 (s, 1H) and 3.68 (s, 3 H), respectively. The spectrum displayed singlet at 1.28 (s, 6H) and 1.81 (s, 3H) showing the presence of three methyl groups. In addition, a triplet peak at 0.89 (t, 3H) revealed the

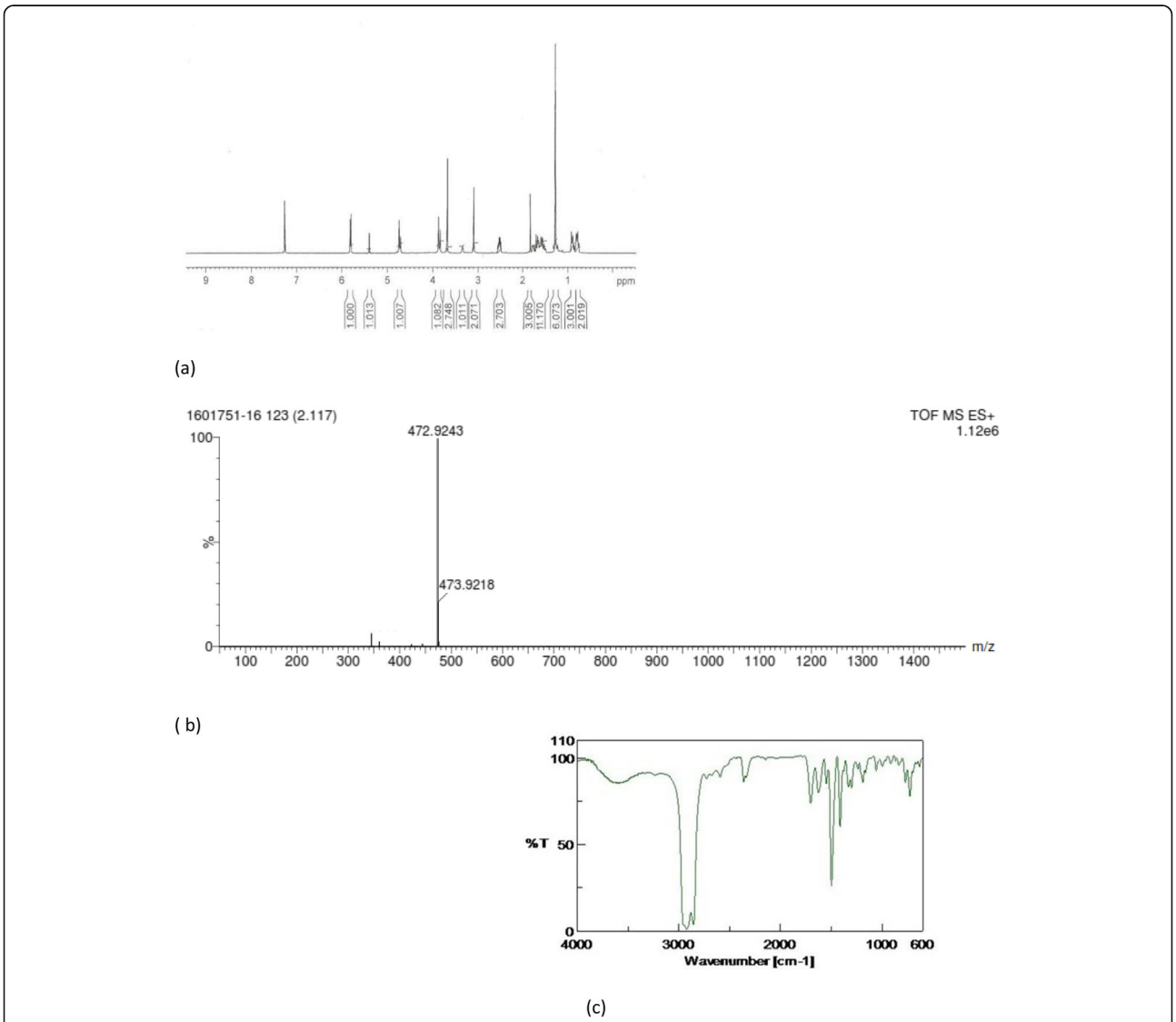

Fig. 1 The spectra of the newly isolated compound **1** from *E. cotinifolia*: **a** ^{1}H-NMR, **b** mass spectra, **c** infra-red

Table 6 ^{1}H NMR in table format

Sl. No.	Chemical shift values	Position of proton in the molecule
1	5.80 (d, 1H)	1
2	5.40 (s, 1H)	2
3	4.73	3
4	3.86	4
5	3.68 (s, 3H)	5
6	3.35 (bs, 1H)	6
7	3.10 (s, 2H)	7
8	2.52 (m, 3H)	8
9	1.83 (s, 3H)	9
10	1.50–1.80 (bm, 12H)	10
11	1.28 (s, 6H)	11
12	0.90 (t, 3H),	12
13	0.80 (m, 2H)	13

Table 7 ^{13}C NMR in table format

Sl. No.	Chemical shift values	Position of carbon in the molecule
1	190	1
2	164	2
3	159	3
4	146	4
5	139	5
6	130	6
7	125	7
8	75	8
9	73	9
10	63	10
11	50	11
12	46	12
13	45	13
14	44	14
15	40	15
16	39	16
17	38	17
18	36	18
19	35	19
20	32	20
21	27	21
22	26	22
23	25	23
24	24	24
25	23	25
26	21	26
27	14	27

presence of another methyl group which is linked to aliphatic carbon chain. The one singlet at 5.40 (s, 1H) and doublet at 5.80 (d, 1H) were due to the presence of olefin protons in the compound. The ^{13}C NMR of compound **1** recorded the presence of 27 signals, including three peaks at δ 159, 164, and 190, which were assigned to the carbonyl group. The mass spectrum of **1** displayed a molecular ion peak at m/z 472 corresponding to its molecular formula $C_{28}H_{40}O_6$.

The genus *Euphorbia* contains the well-known diterpenoids such as jatrophene, lathyrane and myrisinane tigliane, ingenane, daphnane, segetane sesquiterpenoids, flavonoids (ruten kaempferol, myricetri, quercetin derivatives), volatile compounds (terpinene, linalool, α-terpinol), tannins (euphorbins), triterpenoids (lupeol, betulin), and phytosterols (β-sitosterol). These constituents were isolated from different parts of the *Euphorbia* species [4]. Many of the euphorbia compounds have been examined for their toxicity or their potential activity [26]. The literature review of *E. cotinifolia* has revealed the isolation and characterization of new ingenol esters from methanolic extract [10], which possess piscicidal constituents, two new ellagi tannins, and a trigalloylglucoseyl kaempferol from *E. cotinifolia* extract possessing antitumor and antioxidant activity indicating the potential of *E. cotinifolia*. The study by Runyoro and team [27] determined the antifungal activity and brine shrimp lethality of the latex, methanolic leaf, and stem bark extracts of *Euphorbia cotinifolia* and leaves extracts inhibited *Trichophyton mentagrophytes*, *Trichophyton rubrum*, and *Aspergillus niger* with inhibition zones of 12, 17, and 15 mm, respectively, and 9 mm for both fungi with stem bark extracts, while the MICs ranged from 2.5 to 5 mg/ml. In the present study, biologically active diterpene was isolated and the structures were elucidated.

Conclusion

In the present study, biologically active diterpene was isolated and the structures of the new diterpenoids isolated from *E. cotinifolia* were closely related to an ingenol ester in which the alkyl chain is differing in the present structure of active compound of methanol extract. Terpenoids are made up of isoprene units and mode of action of terpenes is not fully understood, but it is speculated to involve in membrane disruption by their liphophilic compound. Polycyclic diterpenoids with tigliane (phorbol esters), ingenane (ingenol esters), jatrophene, and laryprane skeletons are among the most studied diterpenoids isolated from *Euphorbia* species. Thus, in the present study, a terpenoid from *E. cotinifolia* has been reported with an antibacterial activity which could be a source of novel active molecules for drug development.

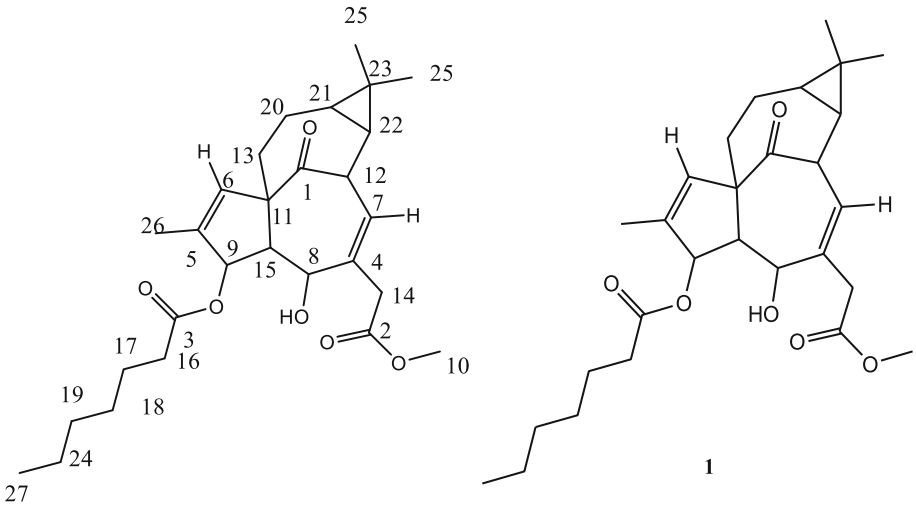

Fig. 2 Structures of newly isolated Compound **1** of *E. cotinifolia*

Currently, there is an increasing interest of plant-based or herbal medicine throughout the world. Exploitation of naturally available compounds from plants, which regulates the growth of undesirable microorganisms, would be a realistic and ecologically sound method for plant protection and will have a prominent role in the development of future commercial drugs.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s43094-020-00160-9.

Additional file 1.

Abbreviations

MTCC: Microbial Type Culture Collection; NA: Nutrient agar; MIC: Minimum inhibitory concentration; ECMF: *Euphorbia cotinifolia* methanol fractions; CFU: Colony forming units; TTC: 2, 3, 5-triphenyl tetrazolium chloride; UV: Ultraviolet; H NMR: Proton nuclear magnetic resonance; C NMR: Carbon nuclear magnetic resonance; TMS: Tetramethysilane; MS: Mass spectra; IR: Infra-red

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Plant authentication

The taxonomists in the Department of studies in Botany, University of Mysore have identified the plant. A voucher specimen of the plant has been deposited in the Herbarium, Department of Studies in Botany, University of Mysore, Mysore. India and voucher /specimen number is assigned as MGBH01.

Authors' contributions

BJL conceived, designed, and performed the above research work. KNA contributed reagents/materials/analysis tools and designed the experiments. KAR analyzed the data. All authors have read and approved the manuscript

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Availability of data and materials

All data and materials are available upon request

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

No competing interests to declare.

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