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In vitro antioxidant, anti-inflammatory, and anticancer activities of methanolic extract and its metabolites of whole plant *Cardiospermum canescens* Wall

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Abstract

Background: Traditionally, the whole plant of *Cardiospermum canescens* has wide applications in the management of oxidative stress and inflammation in Africa and Asia. The present study investigated the antioxidant, anti-inflammatory, xanthine oxidase (XO) inhibitory, and anticancer activities of metabolites present in the crude methanolic extract of whole plant *C. canescens* (**CCE**).

Results: Chemical examination of **CCE** revealed the presence of six known compounds (**1**–**6**). From the results of in vitro studies, it can confirm that **CCE** exhibited notably inhibition of DPPH and superoxide free radicals, along with COX-1, COX-2, 5-LOX, and XO enzymes. Compounds **2** and **3** showed significant inhibition of DPPH and superoxide free radicals. Also, compound **2** exhibited good inhibition of COX-1 and COX-2 enzyme with IC_{50} of 87.0 and 88.0 μg/mL. Furthermore, **CCE** exhibited significant inhibition of 5-LOX and XO enzymes with IC_{50} of 42.5 and 56.0 μg/mL, respectively, while standard with IC_{50} of 42.5 and 56.0 μg/mL, respectively. Among the test series of cancer cell lines, compounds **2**, **3**, and **CCE** showed a significant percentage of cell growth lysis of DLD-1 with IC_{50} values of 52.5, 72.5, and 32.5 μg/mL, respectively. Besides, all the metabolites and **CCE** showed a very weak degree of specificity against NHME, indicates less toxicity to normal cells.

Conclusion: To conclude, the results of the present study indicated that the methanolic extract from the whole plant of *C. canescens* displayed antioxidant activity by inhibiting DPPH and superoxide free radicals; anti-inflammatory effects by regulating enzymes COX-1, COX-2, 5-LOX, and XO; and anticancer activity by inhibiting the growth of MCF-7, DLD-1, HeLa, and A549. These activities can link to natural active compounds **2** and **3**. This study supports the traditional uses of the root of *C. canescens*. These data findings suggest that *C. canescens* can be a promising natural source of biological medicines for oxidative stress, inflammation, gout, and cancer.

Keywords: *Cardiospermum canescens*, DPPH, Superoxide, Cyclooxygenase, 5-Lipoxygenase, Xanthine oxidase, Sulforhodamine B assay

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Background

Cardiospermum is the sole genus of the tropical family Sapindaceae, which records in the flora of Africa and Asia. *Cardiospermum* genus comprises about 30 species across the globe; most of them are reported to be existing in India. Among these, *Cardiospermum canescens* is a climber, commonly termed as "Love in a puff" and "Balloon plant." [1] In the folklore of India and some other Asian countries, the whole plant of *Cardiospermum* species has been used in the treatment of swelling and tumors. Especially, the tribes of Africa and Asia used the whole plant of *Cardiospermum* in the management of rheumatoid arthritis [1, 2]. Also, in the rural areas of Tamil Nadu state, India, the whole plant is used as a leafy vegetable for daily consumption [2, 3].

Biologically, the whole plant of *C. canescens* reported for arthritis, lumbago, neuropathy, fever, stiffness of limbs, piles, nervous disorders, neuropathy, diaphoretic, snake bites, laxative, diuretic, emmenagogue, and mucilaginous [4]. Earlier, a series of phytochemical constituents, namely flavonoids, triterpenoids, saponins, carbohydrates, proteins, alkaloids, and tannins was identified from *C. canescens* [4, 5]. To date, antioxidant, antibacterial, hepatoprotective effects, and cytotoxicity activities were investigated on *C. canescens* [1, 3, 5, 6]. Besides, no chemical investigation has attempted to evaluate the chemical constituents of *C. canescens*. So, the present research study mainly aimed to analyze the chemical composition of the crude methanolic extract of whole plant *C. canescens* (**CCE**) employing chromatography and to monitor antioxidant, xanthine oxidase (XO) inhibitory, anti-inflammatory, and anticancer activities of isolated metabolites and **CCE**.

Methods

Plant material

The whole plant of *Cardiospermum canescens* Wall (Family: Sapindaceae) was collected in Tirupathi Seshachalam hills, Tirupati, Andhra Pradesh, India, in February 2019. Dr. K. Madhava Chetty of Faculty of Sri Venkateswara University (SVU), Tirupati, Andhra Pradesh, India, has authenticated the sample and a voucher specimen with accession number PS-2019-225 which has been deposited at the Department of Botany, SVU, Tirupati, Andhra Pradesh, India.

Reagents

Diclofenac, 5-lipoxygenase (5-LO), trichloroacetic acid (TCA), xanthine, and xanthine oxidase (XO) were purchased from Sigma Aldrich (USA). Cyclooxygenase 1 and Cyclooxygenase 2 (COX-1 and COX-2; no. 560131) purchased from Cayman (USA). Other chemicals were of analytical grade.

Extraction and isolation of compounds from whole plant *C. canescens*

The whole plant *C. canescens* (1.0 kg) was dried and powdered and extracted thrice with methanol (96%) at 25 °C (3 times × 3 days/time). The obtained fractions were evaporated under vacuum to obtain a methanolic extract of *C. canescens* (**CCE**, 45 g, 4.5%w/w).

The **CCE** extract (50 g) was exposed to silica gel (#100-200) column chromatography (CC) through a step gradient of hexane/ethyl acetate solvent, which yielded five main fractions (FI–FV). FI (2 g) subjected to CC (#100-200) against *n*-hexane/ethyl acetate (step gradient) yielded **1** (110 mg) as a yellowish solid. By using CC (#100-200), FII (2.5 g) with dichloromethane/ethyl acetate solvent system (step gradient) obtained **2** (115 mg) as a pale yellow solid, and **3** (110 mg) as a white powder. Similarly, FIII (800 mg) yielded **4** (80 mg) as a grayish solid, FIV (1.0 g) yielded **5** (100 mg) as a greenish semi-solid, and FIV (1.2 g) yielded **6** (120 mg) as a pale brownish solid.

Antioxidant activity

DPPH assay

The metabolites (**1**–**6**) and **CCE** were subjected to 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay [7] in triplicate. Known concentrations of the sample added 0.004% DPPH in methanol. After that, it was incubated at 37 °C for half an hour and recorded for absorbance at 517 nm against the blank using UV-visible spectrophotometry (Spectra MAX plus 384, USA).

Superoxide radical scavenging assay

The metabolites (**1**–**6**) and **CCE** were subjected to scavenging assay of superoxide [8] in triplicate. Known concentrations of the sample added 1 mL of a standardized solution containing 50 μM NBT + 73 μM NADH + 15 μM of PMS in phosphate buffer (pH 7.4) and incubated for 30 min. After that, absorbance was recorded at 562 nm against the blank.

In vitro assays of anti-inflammatory activity

Cyclooxygenase (COX-1 and COX-2) inhibitory assay

By COX inhibitor screening assay kit (Cayman Chemical Company, MI), metabolites (**1**–**6**), and **CCE** were estimated for inhibitory activities of COX [9]. Diclofenac and different concentrations of extracts were used as inhibitor sources. The prostaglandin 2α amount obtained from COX-1 and COX-2 employing substrate arachidonic acid was estimated by using the enzyme immunoassay (EIA) of the same kit. This inhibitory assay performed thrice and their IC_{50} values attained by analysis of linear regression.

5-Lipoxygenase inhibitory assay

The 5-lipoxygenase (5-LOX) activity [10] on linoleic acid was partly disrupted by using a series of metabolites (**1**–**6**) and **CCE** concentrations. By the end of the reaction time, the absorbances of reaction mixtures were determined at 234 nm and the IC_{50} was then calculated. This inhibitory assay was performed thrice with Quercetin as a standard.

Xanthine oxidase inhibitory activity assay

The XO inhibitory activity [11] determined according to a described method with slight modifications. Briefly, to 10 μl of 5 mM xanthine (substrate), 470 μl of sodium phosphate buffer of pH 8.0, 10 μl of the sample (metabolites (**1**–**6**) and **CCE**) was dissolved in DMSO, and 10 μl of enzyme XO was added and mixed in the well, then incubated for 5 min at 25 °C. Later, absorbance was noted at 295 nm. Allopurinol and DMSO were used as standard and control. This inhibitory assay was performed thrice, and their IC_{50} values were obtained by plotting concentration against respective percentage inhibition.

In vitro anticancer assay

Sulforhodamine B colorimetric assay

The in vitro anticancer activity of metabolites (**1**–**6**) and **CCE** was determined by Sulforhodamine B (SRB) assay [12] using four cancer cell lines—MCF-7 (Breast), DLD-1 (Colon), HeLa (Cervical), and A549 (Lung)—and one normal human cell line—normal human mammary epithelial (NHME) (provided by National Centre for Cell Science, Pune). All cell lines were maintained and sampling was prepared according to the procedures of Tatipamula et al. [12]. To 190 μL, screened ideal cell suspension was added to known concentrations of the sample and incubated for 3 h at 37 °C with 90% relative humidity and in the presence of 5% CO_2. After that, 100 μL cold TCA was added and again incubate for 1 h at 4 °C. Then, the entire 96-well plate is washed and air-dried at 25 °C. Then, add 100 μL SRB solution (0.057%); after 30 min, rinse with 1% CH_3COOH and add 200 μL Tris base (10 mM, pH 10.5) solution; and shake for 5 min; absorbance was measured at 510 nm against a blank (contains the only medium).

Statistical analysis

All in vitro assay test results were note as mean ± SD. A one-way analysis of variance (ANOVA) was followed by a *t* test with $p < 0.05$ measured to be statistically significant.

Results

Chemistry

For the first time, six known compounds (**1**–**6**) were identified from the methanolic extract of *C. canescens* (**CCE**) utilizing chromatographic methods and analyses of spectral data namely ^{1}H NMR, MS, IR, and ^{13}C NMR

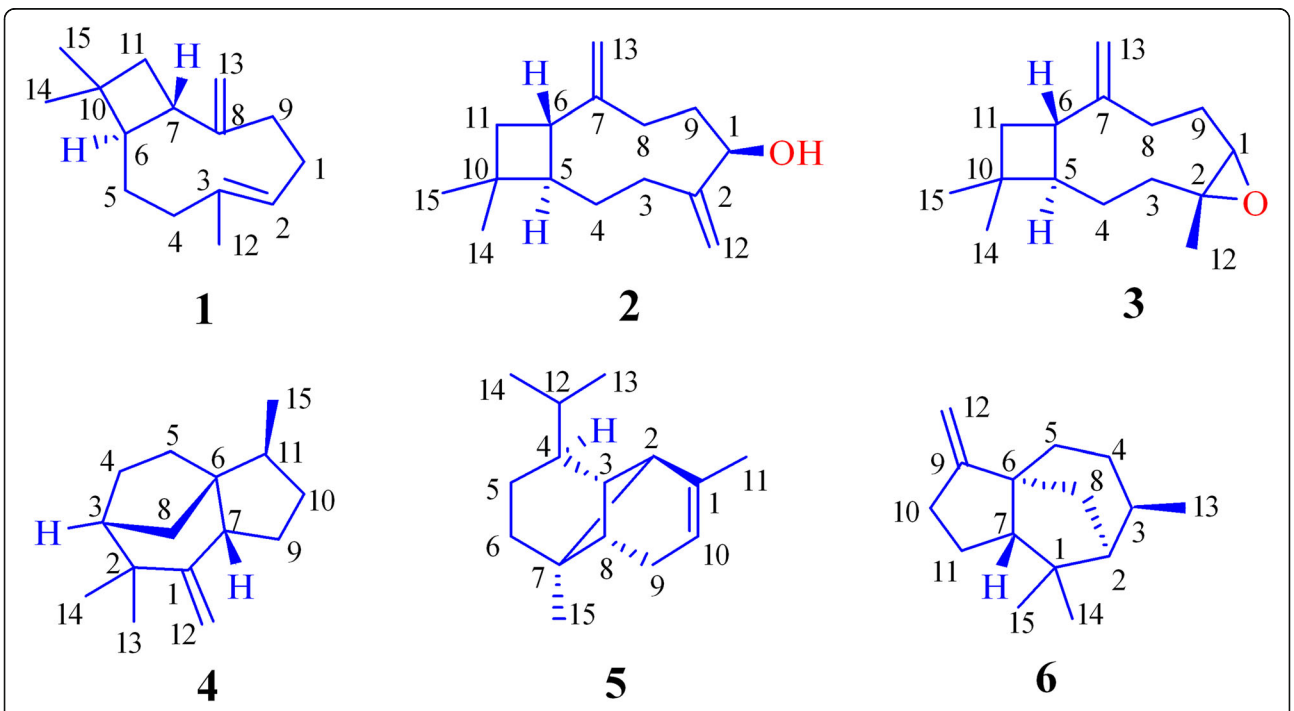

Fig. 1 Known metabolites identified from methanolic extract of *Cardiospermum canescens*

(see supplemental file) and correlated with those reported in the literature (Fig. 1).

Compound **1** [(1R,9S,Z)-4,11,11-trimethyl-8-methylenebicyclo[7.2.0]undec-4-ene] was obtained as a yellowish solid with R_f value of 0.8 in hexane and ethyl acetate (4:1). Mol. formula: $C_{15}H_{24}$. Purity: above 95% pure (based on TLC and NMR observations). ^{1}H NMR (400 MHz, $CDCl_3$): 0.986 (s, 6H), 1.083–1.127 (m, 1H), 1.334–1.378 (m, 1H), 1.685–1.687 (m, 4H), 1.955–1.993 (m, 3H), 1.993–2.157 (m, 4H), 2.510–2.520 (d, 1H, J = 4 Hz), 2.922 (s, 1H), 4.605–4.609 (d, 1H, J = 0.8 Hz), 4.855–4.857 (d, 1H, J = 0.8 Hz), 5.162–5.189 (m, 1H); ^{13}C NMR (400 MHz, $CDCl_3$): 22.21 (C-12), 27.42 (C-5), 29.29 (C-14/15), 30.86 (C-1), 36.82 (C-9/10), 40.13 (C-4), 42.29 (C-11), 51.61 (C-7), 54.52 (C-6), 114.13 (C-13), 126.83 (C-2), 137.31 (C-3), 154.90 (C-8). ESI-MS (positive mode) *m/z*: 205 [M + H$^+$], calcd. *m/z* for $C_{15}H_{24}$: 204.19 [M]. FT-IR (in KBR): 757.67, 830.97, 1037.70, 1088.51, 1129.48, 1160.81, 1239.96, 1342.80, 1420.75, 1455.71, 1503.45, 1581.58, 1619.37, 1657.04, 2864.85, 2926.91, 2958.57 cm^{-1}.

Compound **2** [(1S,5R,9R)-10,10-dimethyl-2,6-dimethylenebicyclo[7.2.0]undecan-5-ol] was obtained as a pale yellow solid with R_f value of 0.4 in chloroform and ethyl acetate (3:7). Mol. formula: $C_{15}H_{24}O$. Purity: above 95% pure (based on TLC and NMR observations). ^{1}H NMR (400 MHz, $CDCl_3$): 0.944 (s, 6H), 1.044–1.082 (m, 1H), 1.295–1.320 (m, 1H), 1.320–1.333 (m, 1H), 1.532–1.543 (m, 2H), 1.613–1.635 (m, 1H), 1.783–1.882 (m, 1H), 1.902–1.926 (m, 1H), 2.013–2.038 (m, 2H), 2.116–2.141 (m, 2H), 2.444–2.504 (m, 1H), 2.992 (s, 1H, -OH), 4.563–4.567 (dd, 2H, J = 0.8 Hz), 4.813–4.817 (dd, 2H, J = 0.8 Hz); ^{13}C NMR (400 MHz, CDCl3): 22.80 (C-4), 24.05 (C-14/15), 28.98 (C-3), 29.56 (C-8), 30.03 (C-9), 31.59 (C-10), 37.06 (C-11), 46.37 (C-6), 49.29 (C-5), 72.64 (C-1), 107.12 (C-12), 108.74 (C-13), 147.5 (C-2), 147.87 (C-7). ESI-MS (positive mode) *m/z*: 221 [M + H$^+$], calcd. *m/z* for $C_{15}H_{24}O$: 220.18 [M]. FT-IR (in KBR): 758.70, 1462.79, 28.55.53, 2921.95 cm^{-1}.

Compound **3** [(1R,4R,10S)-4,12,12-trimethyl-9-methylene-5-oxatricyclo[8.2.0.04,6]dodecane] was obtained as a white powder with R_f value of 0.6 in hexane and ethyl acetate (1:1). Mol. formula: $C_{15}H_{24}O$. Purity: above 95% pure (based on TLC and NMR observations). ^{1}H NMR (400 MHz, $CDCl_3$): 0.960 (s, 6H), 1.160 (s, 3H), 1.239–1.279 (m, 4H), 1.389–1.630 (m, 2H), 1.647–1.691 (m, 2H), 1.898–1.942 (m, 1H), 2.054–2.175 (m, 2H), 2.190–2.205 (m, 1H), 2.461–2.519 (m, 1H), 4.829–4.831 (dd, 2H, J = 0.8 Hz); ^{13}C NMR (400 MHz, CDCl3): 22.87 (C-12), 27.28 (C-4), 32.01 (C-9), 32.22 (C-14/15), 38.07 (C-8), 39.75 (C-10), 40.40 (C-3), 45.22 (C-11), 54.54 (C-6), 59.44 (C-5), 65.15 (C-2), 67.48 (C-1), 116.90 (C-13), 156.03 (C-7). ESI-MS (positive mode) *m/z*: 221 [M + H$^+$], calcd. *m/z* for $C_{15}H_{24}O$: 220.18 [M]. FT-IR (in KBR): 757.43, 1164.22, 1243.88, 1280.23, 1371.49, 1462.62, 1625.60, 2855.31, 2920.71 cm^{-1}.

Compound **4** [(3S,3aS,6R,8aS)-3,7,7-trimethyl-8-methyleneoctahydro-1H-3a,6-methanoazulene] was obtained as a grayish solid with R_f value of 0.4 in hexane and ethyl acetate (1:1). Mol. formula: $C_{15}H_{24}$. Purity: above 95% pure (based on TLC and NMR observations). ^{1}H NMR (400 MHz, $CDCl_3$): 0.893–0.918 (m, 1H), 1.053–1.066 (d, 3H, J = 5.2 Hz), 1.144–1.169 (m, 1H), 1.292–1.298 (m, 1H), 1.386 (s, 6H), 1.433–1.438 (m, 2H), 1.486–1.515 (m, 1H), 1.543–1.574 (m, 2H), 1.568–1.574 (m, 1H), 1.660–1.687 (m, 2H), 2.071–2.106 (t, 2H, J = 6.8, 7.2 Hz), 4.975–4.980 (dd, 2H, J = 0.8 Hz); ^{13}C NMR (400 MHz, $CDCl_3$): 12.17 (C-15), 21.42 (C-9), 23.62 (C-13/14), 23.77 (C-4), 28.97 (C-10), 34.26 (C-5), 35.55 (C-2), 38.14 (C-8/11), 43.31 (C-7), 43.50 (C-3), 52.69 (C-6), 101.50 (C-12), 156.57 (C-1). ESI-MS (positive mode) *m/z*: 205 [M + H$^+$], calcd. *m/z* for $C_{15}H_{24}$: 204.19 [M]. FT-IR (in KBR): 758.59, 1466.21, 2853.30, 2919.84 cm^{-1}.

Compound **5** [(1R,2S,6S,7S,8S)-8-isopropyl-1,3-dimethyltricyclo[4.4.0.02,7]dec-3-ene] was obtained as a greenish semi-solid with R_f value of 0.6 in chloroform and ethyl acetate (3:7). Mol. formula: $C_{15}H_{24}$. Purity: above 95% pure (based on TLC and NMR observations). ^{1}H NMR (400 MHz, $CDCl_3$): 1.044 (s, 3H), 1.057 (s, 3H), 1.061 (s, 3H), 1.215–1.318 (m, 1H), 1.326–1.331 (m, 1H), 1.409–1.434 (m, 2H), 1.488–1.512 (m, 1H), 1.606–1.755 (m, 2H), 1.811–1.871 (m, 2H), 1.912 (s, 1H), 1.913 (s, 3H), 2.050–2.093 (m, 1H), 5.529 (t, 1H, J = 0.8 Hz); ^{13}C NMR (400 MHz, $CDCl_3$): 22.26 (C-5/13/14), 25.62 (C-15), 26.07 (C-11), 30.44 (C-12), 33.80 (C-9), 38.10 (C-6), 39.41 (C-7), 44.84 (C-8), 46.31 (C-4), 47.30 (C-3), 49.31 (C-2), 120.87 (C-10), 143.44 (C-1). ESI-MS (positive mode) *m/z*: 205 [M + H$^+$], calcd. *m/z* for $C_{15}H_{24}$: 204.19 [M]. FT-IR (in KBR): 757.07, 831.97, 1036.44, 1089.60, 1130.24, 1160.95, 1258.86, 1344.88, 1419.78, 1455.32, 1505.34, 1580.19, 1621.23, 1653.08, 2875.04, 2962.67 cm^{-1}.

Compound **6** [(3aR,6R,7S,8aS)-6,8,8-trimethyl-3-methyleneoctahydro-1H-3a,7-methanoazulene] was obtained as a pale brownish solid with R_f value of 0.4 in chloroform and ethyl acetate (1:1). Mol. formula: $C_{15}H_{24}$. Purity: above 95% pure (based on TLC and NMR observations). ^{1}H NMR (400 MHz, $CDCl_3$): 0.880–0.893 (s, 3H, J = 5.2 Hz), 0.937 (s, 6H), 1.061–1.069 (m, 2H), 1.111–1.166 (m, 1H), 1.175–1.180 (m, 2H), 1.185–1.189 (m, 1H), 1.362–1.368 (m, 1H), 1.387–1.429 (m, 4H), 2.054–2.092 (d, 2H, J = 15.2 Hz), 4.587–4.595 (t, 1H, J = 0.8, 2.4 Hz); ^{13}C NMR (400 MHz, $CDCl_3$): 22.03 (C-13), 26.08 (C-14/15), 29.07 (C-11), 30.59 (C-3), 32.88 (C-4), 35.35 (C-10), 35.44 (C-5), 37.96 (C-8), 46.59 (C-1), 52.74 (C-6), 56.18 (C-2), 60.45 (C-7), 100.53 (C-12), 157.13 (C-9). ESI-MS (positive mode) *m/z*: 205 [M + H$^+$], calcd. *m/z* for $C_{15}H_{24}$: 204.19 [M]. FT-IR (in KBR): 699.83, 805.16, 849.70, 1163.13,

1205.87, 1268.76, 1321.28, 1447.21, 1499.94, 1625.06, 2857.43, 2921.21, 3356.81 cm^{-1}.

Antioxidant activity

Initially, **CCE** was exposed to an initial test against DPPH [7] and superoxide [8] assays, and its IC_{50} values were found to be 60.0 and 62.5 μg/mL, respectively, whereas standard (ascorbic acid) value was 27.8 and 32.1 μg/mL, respectively. Based on the preliminary antioxidant analysis of **CCE**, we subjected its metabolites (**1**–**6**) for antioxidant activity. Among all the tested compounds, only compounds **2** and **3** showed moderate inhibition of DPPH and superoxide free radicals. The concentration of **2** needed for 50% inhibition of DPPH and superoxide free radicals was found to be 169.0 and 180.0 μg/mL, respectively, while **3** with 285.0 and 230.0 μg/mL, respectively (Fig. 2).

Anti-inflammatory activity

The anti-inflammatory effect of metabolites (**1**–**6**) and **CCE** were evaluated based on their inhibitory activities against COX-1 and 2 [9], 5-LOX [10] and XO [11] enzymes, initially at 100 μg/mL concentration. During initially screening, **CCE** showed better inhibitor profile against COX-1, COX-2, 5-LOX, and XO enzymes with 72.50 ± 3.44, 80.80 ± 4.30, 70.67 ± 4.57, and 75.90 ± 4.25 %enzyme inhibition, respectively. Besides, among the metabolites, only compound **2** at 100 μg/mL concentration displayed significant inhibitory profile on particularly COX-1 and 2 enzymes with 58.17 ± 3.67 and 56.50 ± 4.50 %enzyme inhibition, respectively. Hence, the active samples, i.e., compound **2** and **CCE**, are further examined at 25, 50, 75, and 100 μg/mL concentrations with standard drugs (indomethacin, diclofenac, and allopurinol) at 2.5, 5.0, 7.5, and 10.0 μg/mL concentrations. From the obtained results, IC_{50} values calculated by plotting concentration against percentage enzyme inhibition.

The 50% COX-1 enzyme inhibitory concentration required for compound **2** and **CCE** was found to be 87.0 and 45.0 μg/mL, respectively, whereas indomethacin with 4.2 μg/mL (Fig. 3a). Similarly, the IC_{50} values of compound **2** and **CCE** on COX-2 were found to be 88.0 and 63.1 μg/mL, respectively, while indomethacin with 4.2 μg/mL (Fig. 3b). The concentration needed for 50% inhibition of 5-LOX and XO enzymes of **CCE** was determined to be 42.5 and 56.0 μg/mL, respectively, whereas standard drugs (diclofenac and allopurinol) with 2.7 and 2.6 μg/mL, (Fig. 3c, d).

Anticancer activity

Firstly, all the isolated metabolites (**1**–**6**) and **CCE** tested against MCF-7 (breast), DLD-1 (colon), HeLa (cervical), A549 (lung), and normal human mammary epithelial (NHME) cell lines at 100 μg/mL concentration. From the primary screening of SRB assay [12], it noticed that **CCE** showed a prominent degree of specificity against the tested series of cancer cell lines. At 100 μg/mL concentration, **CCE** potently inhibited the growth of DLD-1 and A549 with 81.71 ± 8.53 and 75.26 ± 8.55 %cell death, respectively, than standard drug doxorubicin

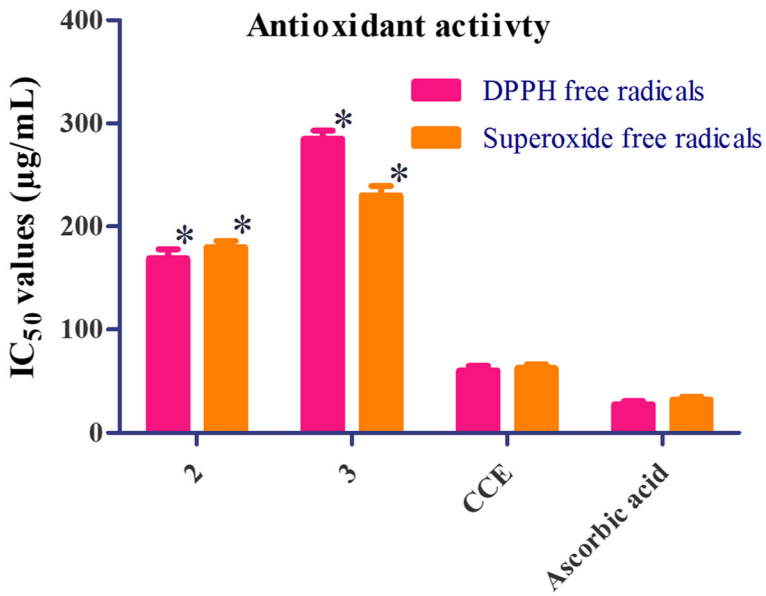

Fig. 2 IC_{50} values of isolated metabolites and methanolic extract of *Cardiospermum canescens* (**CCE**) against DPPH and superoxide free radicals. DPPH, 1,1-diphenyl-2-picrylhydrazyl; $n = 3$, mean ± SD values. Statistical analysis: one-way ANOVA followed by *t* test. *Statistically significant ($p < 0.05$) between free radicals treatment with compounds and extract

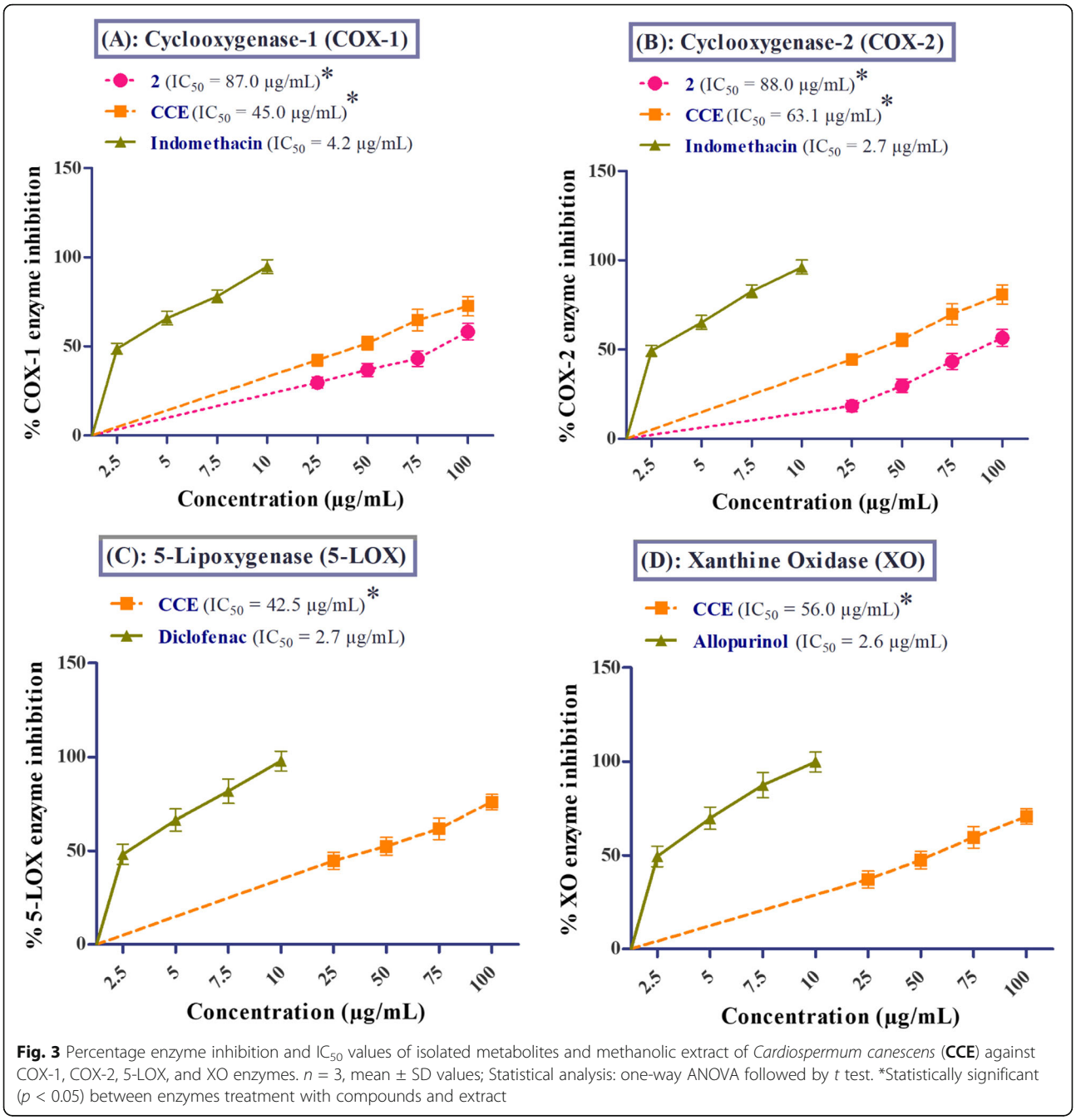

Fig. 3 Percentage enzyme inhibition and IC_{50} values of isolated metabolites and methanolic extract of *Cardiospermum canescens* (**CCE**) against COX-1, COX-2, 5-LOX, and XO enzymes. $n = 3$, mean ± SD values; Statistical analysis: one-way ANOVA followed by *t* test. *Statistically significant ($p < 0.05$) between enzymes treatment with compounds and extract

(10 µg/mL) with 76.17 ± 7.71 and 69.54 ± 5.10 %cell death. Besides, metabolite **2** showed a significant degree of specificity against all the tested panel of cancer cell lines, while compound **3** against only DLD-1 and A549 and compound **1** against only A549.

Secondly, the samples showed above 50% of cell death, examined for further analysis at 25, 50, 75, and 100 µg/mL concentrations with the standard drug (doxorubicin) at 2.5, 5.0, 7.5, and 10.0 µg/mL concentrations. IC_{50} values calculated by plotting concentration against percentage cell growth inhibition. The IC_{50} values of **2** and **CCE** on MCF-7 were found to be 82.0 and 61.5 µg/mL, respectively, whereas doxorubicin with 3.2 µg/mL (Fig. 4a). The concentration of **2**, **3**, and **CCE** needed for 50% cell death of DLD-1 was found to be 52.5, 72.5, and 32.5 µg/mL, respectively, while doxorubicin with 4.2 µg/mL (Fig. 4b). From the results of SRB assay on HeLa, the IC_{50} values of **2** and **CCE** were found to be 96.0 and 78.0 µg/mL, respectively, whereas doxorubicin with 3.8 µg/mL (Fig. 4c). Similarly, the concentration needed for 50% cell death of A549 of 1, 2, 3, and

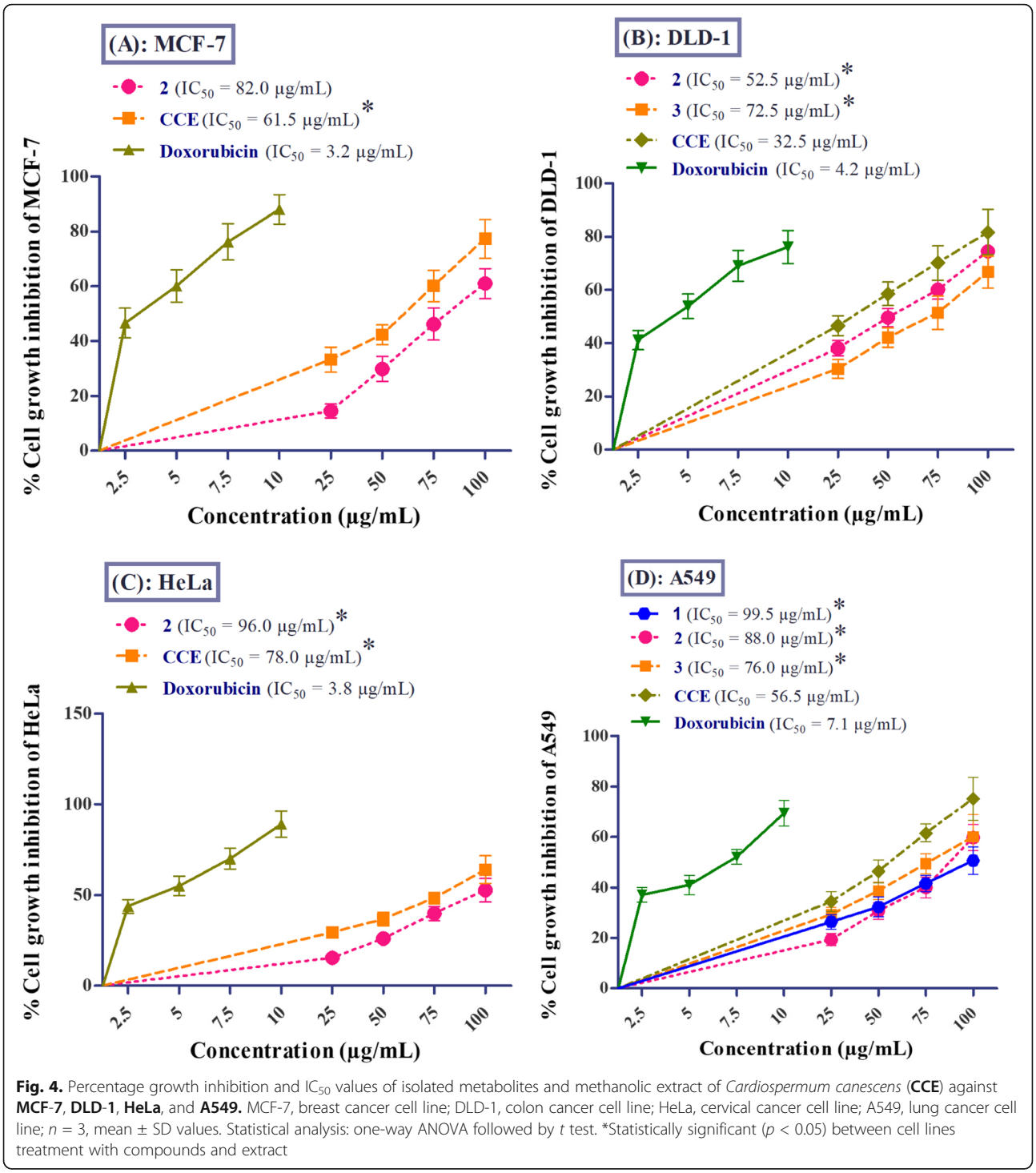

Fig. 4. Percentage growth inhibition and IC_{50} values of isolated metabolites and methanolic extract of *Cardiospermum canescens* (**CCE**) against **MCF-7**, **DLD-1**, **HeLa**, and **A549.** MCF-7, breast cancer cell line; DLD-1, colon cancer cell line; HeLa, cervical cancer cell line; A549, lung cancer cell line; $n = 3$, mean ± SD values. Statistical analysis: one-way ANOVA followed by *t* test. *Statistically significant ($p < 0.05$) between cell lines treatment with compounds and extract

CCE determined to be 99.5, 88.0, 76.0, and 56.5 µg/mL, respectively, while doxorubicin with 3.8 µg/mL (Fig. 4d). Besides, all the isolated compounds (**1**–**6**) and **CCE** showed a very mild degree of specificity against NHME which indicates that the samples are non-toxic to normal human cells.

Discussion

In the present study, six known secondary metabolites (**1**–**6**) were isolated from the methanolic extract of whole plant *C. canescens* (**CCE**). This identification provides new information on the phytochemical profile of *C. canescens*. Also, the biological profile of *C. canescens* justifies its natural aptitude to act against free radicals,

inflammation, and cancer. From the DPPH and superoxide free radical assay, it observed that CCE inhibits them prominently. In the deep enzymatic analysis of anti-inflammation proved that the methanolic extract of whole plant *C. canescens* has potent inhibition of COX-1 and 2, 5-LOX, and XO enzymes. This observation helps to investigate the anticancer ability of *C. canescens*, and the outcomes of SRB assay showed that **CCE** has a significant degree of specificity against MCF-7, DLD-1, HeLa, and A549.

In general, inflammatory activity was caused by an elevated level of prostaglandins and leukotrienes in the body. Cyclooxygenase (COX-1 and 2) and 5-lipoxygenase (5-LOX) enzymes are responsible for the production of prostaglandins and leukotrienes, respectively. Hence, inhibition of COX-1 and 2 by anti-inflammatory drugs such as NSAIDs decrease the production of the prostaglandins, ultimately resulting in the reduction of inflammation, as well as pain [13–15]. Nevertheless, inhibition of particularly prostaglandins can cause activation of 5-LOX pathway alternative, which results in the elevation of pro-inflammatory and gastro-toxic leukotrienes production. Therefore, drugs that inhibit both COX and 5-LOX (dual inhibitors) reduce leukotrienes and prostaglandin production and completely inhibit inflammation. Also, by using dual inhibitors, the adverse effects on the cardiovascular system caused by selective COX-2 inhibitors (coxibs) will be reduced [16–20]. The results of the present study showed that **CCE** acted as a dual inhibitor and effectively inhibited both COX and 5-LOX enzymes, whereas compound **2** prominently inhibit particularly COX-1 enzyme.

Generally, uric acid formed from purines xanthine and purines hypoxanthine in the presence of an enzyme called xanthine oxidase (XO). Excess deposition of uric acid in the joints of the human body leads to severe joint pains, which is well known as gout (painful inflammation) [21, 22]. Furthermore, XO is a good biological source for oxygen-containing free radicals that damage living tissues and cause-related inflammatory diseases like aging, cancer, and atherosclerosis. Hence, XO inhibitors play a vital role in diagnosis, not only gout but also related inflammatory diseases [23–25]. This study suggests that only **CCE** possesses XO inhibitory activity that might be helpful in the management of gout and other inflammatory-related diseases.

Many scientists reported that chronic inflammation is the key causative factor in various types of cancers. Usually, the longer the existence of the inflammation, the greater the risk of cancer. In general, chronic exposure to inflammatory mediators, which include free radicals, cytokines, COX-1, COX-2, and 5-LOX, leads to a sharp rise in mutagenesis, cell proliferation, and oncogene activation eventually leading to the proliferation of cells which lost control over normal growth [26–31]. So, as metabolites and **CCE** showed good anti-inflammatory properties, we further investigated their anticancer abilities using the SRB assay. From the outcomes, it is justified that compound **2**, **3**, and **CCE** have an ability to lysis the cells of MCF-7, DLD-1, HeLa, and A549. Also, all the metabolites and **CCE** are less toxic towards the human cell lines.

In the folklore, the whole plant of *C. canescens* has applications in managing acute arthritis, rheumatism, inflammation, and cancer in India. The present study scientifically justifies the uses of the *C. canescens* as an antioxidant, anti-inflammatory, and anticancer agent in traditional medicine. The major phytoconstituents in **CCE** were identified as **1**–**6**. Thus, these active constituents might be responsible for the biological activities of **CCE**.

Conclusion

To conclude, the results of the present study indicated that the methanolic extract from the whole plant of *C. canescens* displayed antioxidant activity by inhibiting DPPH and superoxide free radicals; anti-inflammatory effects by regulating enzymes COX-1, COX-2, 5-LOX, and XO; and anticancer activity by inhibiting the growth of MCF-7, DLD-1, HeLa and A549. The key metabolite responsible for in vitro activities were claimed to be compound **2** and **3**. The results provide evidence that supports the traditional uses of the whole plant of *C. canescens*. Also, these findings suggest that the plant of *C. canescens* can take an account as a good natural source of remedial medicine for alleviating pain and cancer. However, an in-deep chemical and biological examination is further required to use the natural source (i.e., *C. canescens*) in the management of oxidative stress conditions, inflammation, gout, and cancer.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s43094-020-00028-y.

Additional file 1: Figure S1. 1H NMR (400 MHz, $CDCl_3$) of compound **1**. **Figure S2.** 13C NMR (400 MHz, $CDCl_3$) of compound **1**. **Figure S3.** ESI-MS (positive mode) of compound **1**. **Figure S4.** FT-IR of compound **1**. **Figure S5.** 1H NMR (400 MHz, $CDCl_3$) of compound **2**. **Figure S6.** 13C NMR (400 MHz, $CDCl_3$) of compound **2**. **Figure S7.** ESI-MS (positive mode) of compound **2**. **Figure S8.** FT-IR of compound **2**. **Figure S9.** 1H NMR (400 MHz, $CDCl_3$) of compound **3**. **Figure S10.** 13C NMR (400 MHz, $CDCl_3$) of compound **3**. **Figure S11.** ESI-MS (positive mode) of compound **3**. **Figure S12.** FT-IR of compound **3**. **Figure S13.** 1H NMR (400 MHz, $CDCl_3$) of compound **4**. **Figure S14.** 13C NMR (400 MHz, $CDCl_3$) of compound **4**. **Figure S15.** ESI-MS (positive mode) of compound **4**. **Figure S16.** FT-IR of compound **4**. **Figure S17.** 1H NMR (400 MHz, $CDCl_3$) of compound **5**. **Figure S18.** 13C NMR (400 MHz, $CDCl_3$) of compound **5**. **Figure S19.** ESI-MS (positive mode) of compound **5**. **Figure S20.** FT-IR of compound **5**. **Figure S21.** 1H NMR (400 MHz, $CDCl_3$) of compound **6**. **Figure S22.** 13C NMR (400 MHz, $CDCl_3$) of compound **6**. **Figure S23.** ESI-MS (positive mode) of compound **6**. **Figure S24.** FT-IR of compound **6**. **Table S1.** Percentage inhibition and IC_{50} values of **1-6** and **CCE**

against DPPH and superoxide free radicals. **Table S2.** Percentage inhibition and IC_{50} values of **1-6** and **CCE** against Cyclooxygenase (COX-1 & 2), 5-lipoxygenase (5-LOX) and Xanthine oxidase (XO) enzymes. **Table S3.** Percentage growth inhibition of **1-6** and **CCE** on four different cancer cell lines and one normal human cell line.

Abbreviations

5-LOX: 5-Lipoxygenase; A549: Lung cancer cell line; CC: Column chromatography; CCE: Crude methanolic extract of whole plant *Cardiospermum canescens*; COX-1: Cyclooxygenase 1; COX-2: Cyclooxygenase 2; DLD-1: Colon cancer cell line; DMSO: Dimethyl sulfoxide; DPPH: 1,1-Diphenyl-2-picrylhydrazyl; HeLa: Cervical cancer cell line; MCF-7: Breast cancer cell line; NHME: Normal human mammary epithelial; NMR: Nuclear magnetic resonance; TCA: Trichloroacetic acid; TLC: Thin-layer chromatography; XO: Xanthine oxidase

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Plant source

The whole plant of *Cardiospermum canescens* Wall (Family: Sapindaceae) was collected in Tirupathi Seshachalam hills, Tirupati, Andhra Pradesh, India, in February 2019. Dr. K. Madhava Chetty of Faculty of Sri Venkateswara University (SVU), Tirupati, Andhra Pradesh, India, has authenticated the sample and a voucher specimen with accession number PS-2019-225 was deposited at the Department of Botany, SVU, Tirupati, Andhra Pradesh, India.

Studies involving plants

As per the local and national guidelines and legislation and the required or appropriate permissions and/or licenses for the study.

Authors' contributions

AK: Research scholar who carried isolation and biological evaluations and was a major contributor in writing the manuscript. GSV: Supervisor who guided in isolation and characterization of the secondary metabolites. AVSS: Professor in pharmacology who proposed and monitored the pharmacological studies. All authors have read and approved the manuscript.

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Not applicable

Consent for publication

Not applicable

Competing interests

No conflict of interest between any of the authors.

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