



## ORIGINAL ARTICLE

# Unveiling the anticancer, antimicrobial, antioxidative properties, and UPLC-ESI-QTOF-MS/ GC-MS metabolite profile of the lipophilic extract of siam weed (*Chromolaena odorata*)



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## KEYWORDS

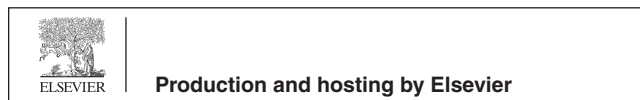
*Chromolaena odorata* lipophilic extract;  
Phytochemical profiling;  
UPLC-QTOF-MS;  
Antioxidant activity;  
Anticancer activity;  
Nutraceuticals

**Abstract** There has been growing interest in the biological and pharmacological potential of *C. odorata* in recent times. While a number of reports had highlighted the beneficial attributes of various extracts prepared from *C. odorata*, the biological properties of the lipophilic extract remained unexplored. This work aimed at examining the anticancer, antimicrobial and antioxidant properties of *C. odorata* aerial part lipophilic extract (COLE). The results revealed that COLE possessed potent and selective anticancer properties against human breast and colon cancer cells. The extract presented strong cytotoxic activity against MCF-7 cells (IC<sub>50</sub> of 9.18 µg/mL), MDA-MB-231 (IC<sub>50</sub> of 12.19 µg/mL), HT-29 (IC<sub>50</sub> of 19.48 µg/mL), and HCT-116 cells (IC<sub>50</sub> of 14.12 µg/mL). Meanwhile, COLE was found to be non-toxic at the concentrations tested against normal (HEK-293 and CCD-841 CoN) cells. In addition, COLE demonstrated significant antibacterial activity against *B. cereus* (MIC value of 0.157 mg/mL), and moderate activity against *L. monocytogenes* (MIC value of 0.63 mg/mL) and *E. coli* (MIC value of 1.06 mg/mL). Furthermore, COLE effectively scavenged free radical species, including ABTS<sup>+</sup> (IC<sub>50</sub> of 46.80 µg/mL), DPPH (IC<sub>50</sub> of 27.78 µg/mL), and

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superoxide anion (IC<sub>50</sub> of 64.07 µg/mL), likely due to the high phenolic (262.48 mg gallic acid equivalent/g) and flavonoid content (70.20 mg quercetin equivalent/g). UPLC-MS analysis revealed the putative identities of 85 secondary metabolites whereas the GC-MS analysis detected 36 volatile constituents in COLE. Also, it was revealed that terpenes and phenolic compounds were the predominant specialized metabolites present in COLE, and putatively responsible for the remarkable biological properties. Taken together, these results revealed the richness of *C. odorata* lipophilic extract in terms of its metabolite profile and biological activities. In conclusion, COLE is worthy of further investigation to take advantage of its good anticancer, antioxidative and antimicrobial properties in pharmacological or nutraceutical application.

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## 1. Introduction

Cancer is a leading cause of global morbidity and mortality, accounting for a massive 10 million deaths worldwide in 2020 (Sung et al., 2021). For most countries, cancer remains the first or second cause of death and represents a major hindrance in increasing life expectancy in every country (Bray et al., 2021). According to recent estimates, about 19.3 million new cases were recorded with female breast cancer and colon cancer representing the first and the third most commonly diagnosed cases (Sung et al., 2021). A number of therapeutic strategies are frequently deployed to combat these cancers, including hormone therapy, radiation therapy, chemotherapy or various combinations of the afore-mentioned. Despite recorded advances in screening and therapeutic development, achieving successful anticancer treatment outcomes remain a challenge. Increasing incidence of high tumor relapse and recurrences, anticancer drug resistance, as well as drug-induced adverse side effects have been highlighted as contributing factors limiting therapeutic success (Skarkova et al., 2019). Thus, it is necessary to continue the search for more effective, safe, and affordable therapeutic alternatives.

Over the years, natural products of plant origin continue to attract strong interest for the identification and development of anti-cancer agents. The presence of specialized metabolites such as phenolics, alkaloids, xanthenes, lignans, terpenoids, fatty acids, coumarins, anthraquinones, etc., with almost limitless structural diversity and biological activity afford huge possibilities for plant-based anticancer drug discovery (Hashem et al., 2022). Besides, phytocomplexes such as plant extracts and essential oils could provide improved clinical efficacy via synergism of the individual specialized metabolites and simultaneous effects on multiple pharmacological targets (Gordaliza, 2007; Schmidt et al., 2007). For instance, fermented dried pericarp water extracts of *Camellia japonica* L. reported exhibited anticancer activity against FaDu cells via regulation of IGF1R-2/mTOR pathway (Cho et al., 2021) whereas the *C. japonica* oil and distillate fractions abrogated the spontaneous metastasis of mouse melanoma BL6 cells (Miura et al., 2007). Similarly, a number of reports in literature have noted the cytotoxic properties of *C. odorata* and its chemical constituents against HeLa cervical cancer cells (Nath et al., 2015; Yusuf et al., 2022a), LLC and L-60 cancer cells (Hung et al., 2011), Cal51, and MCF7 breast cancer cells (Kouamé et al., 2013), underscoring its anticancer potentials.

*Chromolaena odorata* (L) R.M. King & H. Rob. is a perennial shrub of neotropical origin (Muniappan et al., 2005). *C. odorata* belongs to Asteraceae family. The plant produces a scrambling thicket and grows to about three meters. It colonizes vacant lands, pastures, disturbed forests, and riverbanks. In many regions of the world including several countries in Africa and Asia, the plant is widely abundant. Furthermore, the ethnomedicinal application of *C. odorata* has been steadily increasing over the previous six decades. Traditionally, *C. odorata* has been applied for wound healing, for treatment of diabetes, stomach ache, urinary tract infections and cancer (Tun et al., 2022). Notable chemical constituents present in *C. odorata* include

flavanones, flavonoids, phenolic acids, terpenoids, chalcones, alkaloids, and fatty acids. Complex mixtures of lipophilic aglycones, viz: chalcones, flavonols, flavanones, and flavones, as well as phenolic acids with powerful antioxidant properties were implicated in the effective wound healing action associated with *C. odorata* (Phan et al., 2001). Eupolin, a herbal remedy based on aqueous extract of *C. odorata* enhanced the proliferation of fibroblast and endothelial cells (Phan et al., 1998). Meanwhile, methyl cellulose encapsulated *C. odorata* methanol extract was found to inhibit the growth of *E. coli* and *S. aureus* (Azmi et al., 2019). The essential oil from *C. odorata* aerial part was found to modulate inflammation via its effect on cyclooxygenase function of prostaglandin-H synthase activity (Bedi et al., 2015). Nonetheless, there had not been any published account on the biological properties *C. odorata* aerial part lipophilic extract prepared without noxious organic solvents.

This work aimed to examine the biological attributes of *C. odorata* lipophilic extract focusing on the anticancer, antimicrobial and antioxidant activities. Furthermore, UPLC-ESI-QTOF-MS and GC-MS analysis were employed to profile the various individual secondary metabolites found in the extract. The results generated from this work could provide for the first time an overview of the secondary metabolites present in the lipophilic extract of *C. odorata* aerial parts as well as delineate its anticancer and antioxidative potential.

## 2. Materials and methods

### 2.1. Preparation of *C. odorata* lipophilic extract

*C. odorata* lipophilic extract was prepared according to the procedure previously described by (Yanping Huang et al., 2022) with minor modifications. Fresh aerial part of *C. odorata* was collected from the botanical garden of the Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat Yai, Songkhla, Thailand, with coordinates of 7°00'39" N and 100°29'49" E. The sample was authenticated by Asst. Prof. Dr. Opeyemi Joshua Olatunji, Faculty of Traditional Thai Medicine, Prince of Songkla University, Hat Yai 90110, Thailand and specimen was deposited at the herbarium with voucher F.N.2 (PSU). The plant sample was air-dried under a shade for five days and then ground into fine powder using WF-10B Thai grinder (Bangkok, Thailand) electric grinder. The powder (50 g) was subsequently extracted with 80 % (v/v) ethanol (1 L) using an overhead stirrer at ambient conditions for 2 h. The mixture was filtered to separate the extract from the residue. The residue was re-extracted following the same procedure. The extracts from the two rounds of extraction were combined and concentrated under vacuum.

using a rotary evaporator at 35 °C to one-fifth of the initial volume. The extract was then kept at 4 °C for 12 h to allow it

to fractionate. Thereafter, the clear hydrophilic upper layer was carefully removed, and the congealed lipophilic bottom layer was collected and further dried in a rotational vacuum concentrator (RVC 2–18 CDplus, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode, Germany) to give a dark green paste of *C. odorata* lipophilic extract (COLE). The percentage yield of COLE obtained in terms of *C. odorata* aerial part dry weight was 5.28 %. COLE was stored in airtight amber vials at 4 °C awaiting further investigations.

## 2.2. Chemical characterization of COLE

### 2.2.1. Uplc-esi-qtof-ms analysis of cole

Qualitative metabolite profile of the individual compounds present in COLE was obtained by UPLC-ESI-QTOF-MS analysis. COLE (40 mg) was dissolved in 1 mL of 70 % (v/v) methanol and vortexed for 10 min. The COLE sample was then centrifuged at 8000 rpm for 10 min and the supernatant was collected. The COLE solution was further filtered through a 0.2 µm nylon membrane syringe filter to obtain a clear solution. This solution was then subjected to UPLC-ESI-QTOF-MS analysis as detailed previously (Eze and Tola, 2020; Yanping Huang et al., 2022). The analysis was performed on Liquid chromatograph-quadrupole time-of-flight mass spectrometer (LC-QTOF MS), 1290 Infinity II LC-6545 Quadrupole-TOF (Agilent Technologies, USA) equipped with MassHunter Workstation Software, Qualitative Analysis Workflow, Version B.08.00 (Agilent Technologies, Inc. USA). The mass spectrometer consisted of a model G6545A MS QTOF, fitted with a Dual AJS ESI ion source and operated in a negative ionization mode at 325 °C drying gas temperature, 13 L/min drying gas flow rate, 35 psig nebulizer pressure, 4000 V capillary voltage, 175 V fragmentor voltage, 750 V OctopoleRFpeak, and 10 eV, 20 eV, and 40 eV fixed collision energies. The LC component was equipped with a model G7129B autosampler, a model G7120A binary pump, a model G7116B column compartment and a model G7117A diode array detector. Sample separation was accomplished with a reversed phase Zorbax Eclipse Plus C18 2.1 × 150 mm, 1.8 µm column (Agilent Technologies, USA) as the stationary phase while the mobile phase consisted of ultrapure water + 0.1 % formic acid (solvent A) and acetonitrile (solvent B). The sample (2.0 µL) was injected and eluted at a flow rate of 0.2 mL/min using a gradient elution of 0.50 min, 100% A; 16.50 min, 0 % A; 17.50 min, 0 % A; 20.00 min, 100 % A; 22.00 min, 100 % A.

### 2.2.2. Gc-ms analysis of cole

GC-electron ionization mass spectrometry (GC-EI-MS) was used to profile the volatile and semi-volatile constituents in COLE. The analysis was performed on GC/MS GC 7890, MSD 5977B (Agilent Technologies, USA) with helium as the carrier gas and Agilent HP-5MS column (30 m × 250 µm × 0.25 µm). The flow rate, pressure, average velocity, holdup time and post run were 1 mL/min, 7.0699 psi, 36.262 cm/s, 1.3789 and 1 mL/min, respectively. One microliter of the sample was injected in split mode. The total run time was 72 min consisting of an initial oven temperature of 40 °C, held for 1 min, increased to 250 °C at 5 °C/min (held for 5 min), again increased further to 320 °C at 5 °C/min (held for 10 min). The tentative identities of the compounds present

in COLE was established with the aid of Wiley 10 and NIST14 (National Institute of Standards and Technology 14 (NIST14) chemical libraries. Match score  $\geq 90$  % was considered acceptable (Yanping Huang et al., 2022).

### 2.2.3. Total phenolic and flavonoid content of COLE

The total phenolic content of COLE was determined using Folin-Ciocalteu assay as previously described. COLE solution (100 µL) or gallic acid (standard) was introduced into 2-mL Eppendorf tubes. Into the sample solution was added 200 µL of 10 % (v/v) Folin-Ciocalteu reagent. The tube was vortexed and incubated for 5 min. Thereafter, 800 µL of Na<sub>2</sub>CO<sub>3</sub> solution was added to the sample, vortexed and incubated for 2 h in the dark. The absorbance of the blue reaction mixture was read at 765 nm. The total phenolic content of COLE was determined from calibration curve prepared using gallic acid and expressed as mg gallic acid equivalent per gram of COLE.

The total flavonoid content was determined using aluminum chloride colorimetric assay with quercetin serving as standard. Succinctly, the reaction mixture consisting of COLE solution or quercetin solution (30 µL), methanol (160 µL), freshly 10 % (w/v) aluminum chloride in methanol (30 µL), 1 M sodium acetate solution (30 µL) and distilled water (850 µL) was vortexed and incubated at room temperature in the dark for 30 min. The absorbance of the solution was recorded at 415 nm. The total flavonoid content was calculated from the calibration curve prepared using quercetin and presented as mg quercetin equivalent per gram of COLE.

### 2.2.4. Total saponin content of COLE

The total saponin content of COLE was estimated by vanillin-sulfuric acid colorimetric assay with asiatic acid serving as standard (Le et al., 2018). Briefly, solution of COLE or asiatic acid (200 µL) was introduced into a screw cap glass test tube. Vanillin solution (8 % w/v in methanol; 200 µL) was added to the sample and vortexed. Then sulfuric acid (72 %; 2 mL) was added into the solution. The solution was vortexed and incubated at 60 °C for 10 min. After cooling at room temperature, the absorbance was read at 544 nm. The total saponin content was calculated from the calibration curve prepared using asiatic acid and expressed as mg asiatic acid equivalent per gram of COLE.

## 2.3. Color analysis of COLE

Hydrophilic and lipophilic fractions of *C. odorata* ethanol extract (5 mg/mL) were prepared in 50% aqueous ethanol and absolute ethanol, respectively. Color analysis of the extracts were performed using total transmittance mode (TTRAN) on HunterLab UltraScan pro (HunterLab, USA). The color parameters *L* (Lightness), *a* (redness to greenness), *b* (yellowness to blueness) were obtained from the sample, taking into account their corresponding blanks, while the hue angle, chroma and total color change  $\Delta E$  were calculated as previous reported (Acero et al., 2019).

## 2.4. Determination of anticancer activity of COLE

### 2.4.1. Cell lines and culture conditions

Human breast cancer (MCF-7: ATCC HTB-22 and MDA-MB-231: ATCC HTB-26), colonic cancer (HT-29: ATCC

HTB-38 and HCT-116: ATCC CCL-247) and non-cancer (human embryonic kidney, HEK-293: ATCC CRL-1573, and human normal colon epithelial, CCD-841 CoN: ATCC CRL-1790) cell lines were procured from American Type Culture Collection (ATCC, Manassas, VA, USA). All cells were cultured in a humidified incubator with 5 % CO<sub>2</sub> at 37 °C and nourished with DMEM (Dulbecco's Modified Eagle Medium) medium (Sigma-Aldrich, USA) supplemented with 10% fetal bovine serum (Gibco, Thermo Fisher, USA) and 100 units/mL of penicillin and 100 µg/mL of streptomycin (Gibco, Thermo Fisher, USA).

#### 2.4.2. *In vitro* cytotoxicity

The cytotoxic activity of COLE was determined via MTT assay according to previous report (Hrubik et al., 2012). Initially, the cells were seeded at a density of  $2 \times 10^4$  cells/well into 96-well microplates and allowed to attach. Thereafter, the cells were exposed to different concentrations of COLE (1 – 800 µg/mL) for 72 h. Negative control consisted of wells treated only with the diluent. After cell viability (%) was calculated, the IC<sub>50</sub> values of COLE on the various cell lines were determined using GraphPad prism 7.0.

#### 2.5. Determination of antimicrobial activity of COLE

The antimicrobial property of the lipophilic (COLE) and hydrophilic (COHE) fractions of *C. odorata* was determined against the following bacteria viz: *Escherichia coli* O157:H7 (ATCC 43890), *Listeria monocytogenes* F2365 (Nelson et al., 2004), and one clinical isolate of *Bacillus cereus* from the culture collection of the Division of Biological, Prince of Songkla University, Hat Yai. Vancomycin hydrochloride and gentamicin sulfate (Sigma-Aldrich Pte Ltd., Singapore) were used as positive control. The antimicrobial activity of the samples was evaluated using broth microdilution assay as previously described (CLSI, 2020).

Pure bacterial inoculums grown in tryptic soy broth (TSB) (Difco, Le Port de claix, France) at early log phase were adjusted to  $10^6$  CFU mL<sup>-1</sup> using MHB (Müller Hinton broth) to obtain bacterial suspensions. The respective bacterial suspensions (100 µL) were introduced into wells of 96-well plates containing serial dilutions of COLE or COHE. The plates were subsequently incubated at 37 °C for 24 h. The lowest concentrations of *C. odorata* sample that completely inhibited bacterial growth was regarded as the minimum inhibitory concentration (MIC) value. In addition, the MBC (minimum bactericidal concentration) values of the samples were also estimated. The MBC values were ascertained using the spot plot technique by seeding 10 µL aliquots from the wells that did not display any microbial growth onto TSA plates followed by incubation for 18 h at 37 °C. The MBC value of each sample is taken from the lowest concentration that showed no microbial growth on the TSA plates. All experiments were performed in triplicates for two independent studies.

#### 2.6. Determination of antioxidant activity of COLE

##### 2.6.1. DPPH radical scavenging activity

The radical scavenging activity of COLE against DPPH was determined spectrophotometrically as previously described by (Lesjak et al., 2018) with minor modifications. Succinctly,

the samples diluted with methanol (100 µL) were added into the wells of 96-well plates. Wells containing only methanol (100 µL) were used as the control. DPPH solution in methanol (0.1 mM, 100 µL) was added to samples. In the blank wells, methanol (100 µL) was added to the samples instead of DPPH solution. Ascorbic acid was used as a reference antioxidant compound. The reaction solutions were allowed to incubate in the dark for 30 min. Thereafter, absorbance was recorded at wavelength of 515 nm. After correcting for the blank, the percentage radical scavenging activity (RSA %) was calculated thusly:

$$RSA (\%) = [(AC - AS)/AC] * 100$$

Where AC was absorbance of the control solution and AS was absorbance of the sample solution. The antioxidant activity was estimated from the curve of RSA vs. concentration, plotted using GraphPad Prism version 7.0, and expressed as IC<sub>50</sub> i.e., the concentration that scavenged the DPPH radical by 50%.

##### 2.6.2. Abts radical scavenging activity

Firstly, the ABTS<sup>+</sup> stock solution was made by mixing 2 mL of ABTS solution (7.4 mM) and 2 mL of potassium persulfate solution (2.6 mM) followed by incubation for 12 h. The working solution was prepared by diluting the stock solution with ethanol to achieve an absorbance value of  $0.70 \pm 0.01$  at 734 nm. Then COLE solution (15 µL) was added into 96-well microplate followed by 180 µL of the ABTS<sup>+</sup> working solution. Ascorbic acid was used as a reference antioxidant compound. After 10 min of incubation, the absorbance was measured at 734 nm. The ABTS<sup>+</sup> radical scavenging activity of COLE was calculated thusly:

$$RSA (\%) = [(AC - AS)/AC] * 100$$

Where AC was absorbance of the control solution and AS was absorbance of the sample solution. The IC<sub>50</sub> value of the sample against ABTS was estimated as described in the DPPH assay supra.

##### 2.6.3. Determination of superoxide anion radical scavenging

Briefly, 2.52 mM nitrobluetetrazolium chloride (50 µL), 624 mM NADH (50 µL) were mixed to generate the superoxide anion. COLE solution (50 µL) with final concentration ranging from 10 to 100 µg/mL, was added to the radical solution. Ascorbic acid was used as a reference antioxidant compound. To initiate the reaction, 120 µg/mL of phenazine methosulfate solution (50 µL) was added to the reaction mixture. After 5 min of incubation at room temperature, absorbance of the solutions were recorded at 560 nm (Sannasimuthu et al., 2018). The radical scavenging activity (RSA) was calculated as follows:

$$RSA (\%) = [(OD control - OD sample)/OD control] * 100$$

The IC<sub>50</sub> value of the sample against superoxide anion was estimated as described in the DPPH assay supra.

##### 2.6.4. Metal chelation activity

The ability of COLE to chelate ferrous ions was estimated using a colorimetric assay with EDTA-Na<sub>2</sub> (ethylenediaminetetraacetic acid disodium salt) serving as positive control (Santos et al., 2017). The reaction mixture consisted of COLE

or EDTA- $\text{Na}_2$  solution (50  $\mu\text{L}$ ), ultrapure water (160  $\mu\text{L}$ ), and 0.30 mM  $\text{FeSO}_4$  solution (20  $\mu\text{L}$ ). After 5 min, 0.8 mM Ferrozine solution (30  $\mu\text{L}$ ) was added. The reaction mixture was incubated for 30 min after which absorbance was read at 562 nm. The metal chelating effect as a percentage was obtained thus:

$$\text{Metal chelating effect (\%)} = [1 - (AS/AC)] * 100$$

Where, AS and AC are the absorbance of the sample and control, respectively.

### 2.7. Statistical analysis

All sample data were analyzed using ANOVA followed by post hoc analysis using Tukey test. The statistical analysis was performed on GraphPad Prism version 7.0 (GraphPad Software, Inc., San Diego, CA). Values with  $p < 0.05$  were considered statistically significant.

## 3. Results and discussion

### 3.1. Chemical composition of *C. odorata* lipophilic extract

Plant bioactives, especially polyphenols such as flavonoids are widely regarded as powerful sources of natural antioxidants

and the health benefits of consuming fruits and vegetables has largely been attributed to these specialized metabolites. Consequently, there has been a consistent interest in identifying new sources rich in bioactive components as well as new approaches for their preparation. Herein, *C. odorata* lipophilic extract (COLE) from the aerial part was prepared in a simple green chemistry approach (Fig. 1). The only organic solvent used in the extraction process was ethanol, which is deemed to be generally recognized as safe (GRAS) in the food industry. This was to ensure that if the extract exhibits desirable attributes, then valorizing it into useful phytomedicinal or nutraceutical products would not be challenging. In particular, the challenge arising from solvent-associated toxicity would not be a serious issue of concern. The crude *C. odorata* extract obtained using food grade ethanol was portioned via cold fractionation into an upper hydrophilic component (COHE) and a lower lipophilic component (COLE). Various classes of specialized metabolites from the *C. asiatica* extracts were initially evaluated via UV-vis spectroscopy and the results are presented in Table 1. The lipophilic extract was found to possess high amounts of total phenolics, flavonoids and saponins. The high phenolic content of COLE was found to be in line with previous reports on *C. odorata* extract (Srinivasa Rao et al., 2010). Interestingly, comparison of the phytochemical content of COLE with the hydrophilic fraction revealed that the COLE contained higher amounts of phytochemicals.

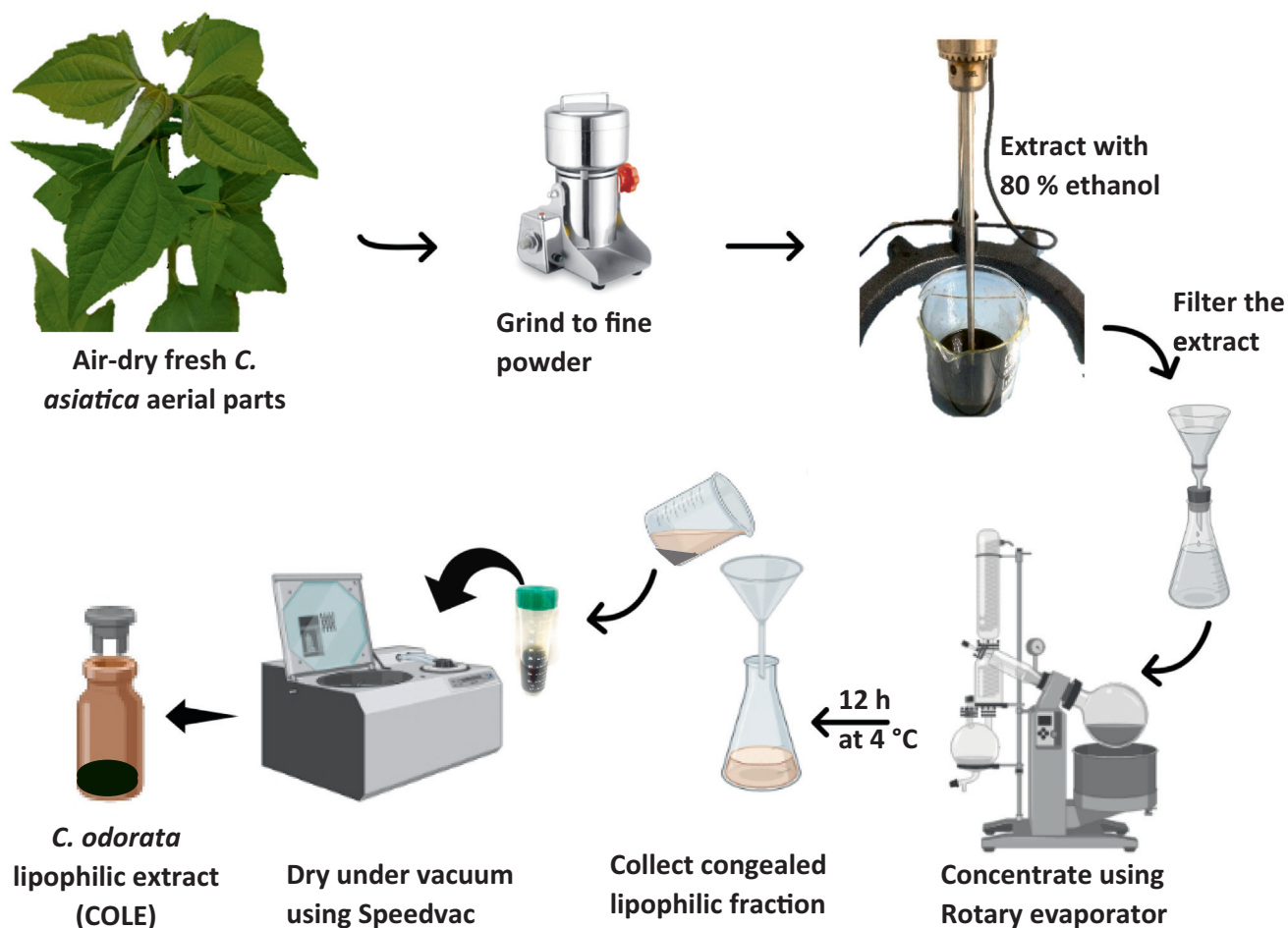


Fig. 1 Schematic representation of the preparation of *C. odorata* lipophilic extract (COLE) from aerial part of the plant.

Besides, both components of *C. odorata* were also different in their physical appearance. The hydrophilic layer was clear orange in appearance while the lipophilic layer presented a dark green appearance. The color of the two samples were examined in terms of the following chromatic parameters viz; *L*, lightness: white/ black (100/0), *a*: red/green (+/-), *b*: yellow/blue (+/-), and  $\Delta E$ , color difference. The *L* values confirmed that that COHE (91.57) was lighter than COLE (16.73). Also, it was observed that the chroma values were markedly different in both fractions. The chroma value of COHE was more than twice that of COLE. Contrariwise, the values for all the colorimetric parameters for COLE, with the exception of *a*, were lower relative to those observed for COHE. This therefore justified the darker appearance COLE.

### 3.1.1. Secondary metabolites profile of *C. odorata* lipophilic extract by UPLC-MS analysis

In this study, an extensive qualitative phytochemical profile of the individual bioactive constituents present in COLE was established via UHPLC-ESI-QTOF-MS analysis in negative ionization mode. Prior preliminary analysis indicated that the negative ionization mode was more sensitive than the positive ionization mode. The total ion chromatogram of the extract in the negative ionization mode is presented in Fig. 2 and features several peaks at different retention times spread across the length of the chromatogram, suggestive of phytoconstituents belonging to different families (Fig. S1). The individual compounds in the lipophilic extract of *C. odorata* that were tentatively identified via QTOF-MS analysis in the negative ionization mode and with an accuracy error below 5 ppm (Table 2). In addition, other parameters that facilitated the

characterization of the bioactive compounds such as retention times (Rt), detected accurate mass ( $[M-H]^-$ ), mass error (ppm), and molecular formula of each chemical component are also provided (Eze et al., 2019).

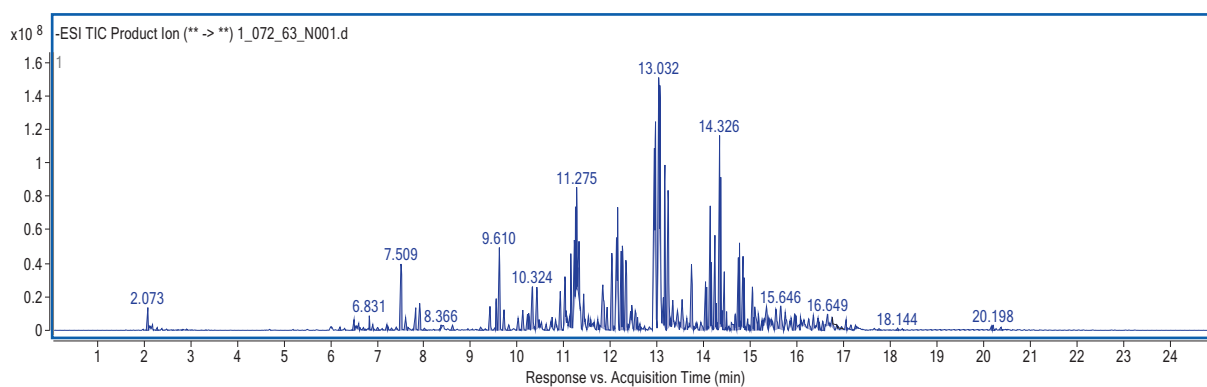
### 3.1.2. Terpene and terpenoid derivatives

It has been previously noted that terpenoids could be vital in the management of cancer (Ben Sghaier et al., 2016; Dahham et al., 2015; Hui et al., 2015), type 1 diabetes via the preservation and rejuvenation of  $\beta$  cells (Liu et al., 2010), and in type 2 diabetes through activation of the transcription factor Nrf2 (Castellano et al., 2013). Also widely reported is the fact that terpenoid compounds could stimulate wound healing and antimicrobial actions via multiple mechanisms (Agra et al., 2015). Some studies have alluded to the presence of terpenoids in *C. odorata* aerial parts, but very few have actually mentioned the individual compounds from this group (Joshi, 2013; Vijayaraghavan et al., 2018). Thus, it was essential to establish the identities of the terpenoid compounds present in *C. odorata* hydrophilic extract. Mass spectral data from COLE revealed the presence of the following putative terpene glycosides; (1*S*,2*R*,4*R*,8*S*)-*p*-menthane-2,8,9-triol 9-glucoside (Rt 6.436), glucosyl (2*E*,6*E*,10*x*)-10,11-dihydroxy-2,6-farnesadienoate (Rt 6.461 min), sonchuioside C (Rt 6.938 min), 3-hydroxy- $\beta$ -ionol 3-[glucosyl-(1  $\rightarrow$  6)-glucoside] (Rt 7.139 min), 10-acetoxyligustroside (Rt 7.315 min), neryl rhamnosyl-glucoside (Rt 7.34 min), kenposide B (Rt 9.147 min). The terpenoids, (24*E*)-3 $\alpha$ ,15 $\alpha$ -diacetoxy-23-oxo-7,9(11),24-lanostatrien-26-oic acid (Rt 13.943 min), (24*E*)-3 $\beta$ ,15 $\alpha$ ,22*S*-triacetoxylanosta-7,9(11),24-trien-26-oic acid (14.733 min), glycosyl-4,4'-diaponeurosporenoate (Rt

**Table 1** Phenolics, saponins contents of COE fractions and color analysis.

Sample	Phenolic and saponin contents			Color analysis			
	Total phenolics (mg gallic acid/g)	Total flavonoids (mg quercetin/g)	Total saponin (mg asiatic acid/g)	L	a	b	$\Delta E$
COHE	189.96 $\pm$ 1.25 <sup>a</sup>	22.82 $\pm$ 0.87 <sup>b</sup>	55.07 $\pm$ 1.28 <sup>c</sup>	91.57 $\pm$ 0.01	-4.07 $\pm$ 0.01	29.38 $\pm$ 0.01	96.25 $\pm$ 0.01
COLE	262.48 $\pm$ 1.66	70.20 $\pm$ 22.60	129.56 $\pm$ 18.39	16.73 $\pm$ 0.03	6.54 $\pm$ 0.03	10.91 $\pm$ 0.02	21.02 $\pm$ 0.04

<sup>a,b,c</sup> These values have been reported in our previous work, see (Eze and Jayeoye, 2021), but were included here for comparison.



**Fig. 2** Total ion chromatogram of secondary metabolites found in *C. odorata* lipophilic extract by UPLC-ESI-QTOF-MS in negative ionization mode.

**Table 2** Chemical profile of compounds present in *C. odorata* lipophilic extract obtained by UPLC-ESI-QTOF-MS analysis.

S/ N	RT (min)	Precursor (m/z)	Accurate Mass	Diff (ppm)	Score (DB)	Formula	Tentative ID
1	2.01	373.0924	374.0999	0.77	97.7	C <sub>19</sub> H <sub>18</sub> O <sub>8</sub>	Quercetagenin 6,7,3',4'-tetramethyl ether
2	2.19	191.0563	192.0636	-1.06	99.43	C <sub>7</sub> H <sub>12</sub> O <sub>6</sub>	Quinic acid
3	4.75	271.0819	272.0891	1.81	98.55	C <sub>12</sub> H <sub>16</sub> O <sub>7</sub>	Arbutin
4	5.25	169.0141	170.0215	0.38	99.56	C <sub>7</sub> H <sub>6</sub> O <sub>5</sub>	Gallic acid
5	5.63	315.0717	270.0737	1.08	98.33	C <sub>12</sub> H <sub>14</sub> O <sub>7</sub>	Phenyl glucuronide
6	5.70	359.0981	360.1054	0.76	99.3	C <sub>15</sub> H <sub>20</sub> O <sub>10</sub>	6'-Methoxypolygoacetophenoxide
7	5.93	323.1345	264.1206	1.13	97.89	C <sub>11</sub> H <sub>20</sub> O <sub>7</sub>	(Z)-2-Methyl-2-butene-1,4-diol 4-O-β-D-Glucopyranoside
8	5.95	479.2491	480.2563	1.5	97.4	C <sub>22</sub> H <sub>40</sub> O <sub>11</sub>	3-O-(α-L-Rhamnopyranosyl-(1 → 2)-α-L-rhamnopyranosyl)-3-hydroxydecanoic acid
9	6.06	353.0877	354.095	0.25	99.86	C <sub>16</sub> H <sub>18</sub> O <sub>9</sub>	Chlorogenic acid
10	6.33	153.0193	154.0266	-0.06	99.89	C <sub>7</sub> H <sub>6</sub> O <sub>4</sub>	3,4-Dihydroxybenzoic acid
11	6.36	285.0615	286.0687	0.51	99.78	C <sub>12</sub> H <sub>14</sub> O <sub>8</sub>	Uralennoiside
12	6.43	349.1863	350.1935	1.59	98.74	C <sub>16</sub> H <sub>30</sub> O <sub>8</sub>	(1S,2R,4R,8S)-p-Menthane-2,8,9-triol 9-glucoside
13	6.46	477.2335	432.2351	1.95	74.39	C <sub>21</sub> H <sub>36</sub> O <sub>9</sub>	Glucosyl (2E,6E,10x)-10,11-dihydroxy-2,6-farnesadienoate
14	6.56	191.0563	192.0636	-0.95	99.46	C <sub>7</sub> H <sub>12</sub> O <sub>6</sub>	Quinic acid
15	6.81	771.1985	772.2058	0.56	98.54	C <sub>33</sub> H <sub>40</sub> O <sub>21</sub>	Quercetin 3-glucosyl-(1 → 2)-[rhamnosyl-(1 → 6)-galactoside]
16	6.93	431.1925	386.1942	-0.3	81.48	C <sub>19</sub> H <sub>30</sub> O <sub>8</sub>	Sonchuionoside C
17	6.96	299.0771	300.0844	0.44	99.64	C <sub>13</sub> H <sub>16</sub> O <sub>8</sub>	Salicylic acid β-D-glucoside
18	7.01	755.2038	756.2109	0.5	98.85	C <sub>33</sub> H <sub>40</sub> O <sub>20</sub>	Kaempferol 3-sophoroside 7-rhamnoside
19	7.03	433.2079	388.2103	-1.44	78.62	C <sub>19</sub> H <sub>32</sub> O <sub>8</sub>	5α,6α-Epoxy-7E-megastigmen-3β,9ξ-diol 9-glucoside
20	7.06	415.1245	416.1318	0.12	98.73	C <sub>18</sub> H <sub>24</sub> O <sub>11</sub>	2-Hydroxybenzaldehyde O-[xylosyl-(1 → 6)-glucoside]
21	7.13	593.2812	534.2674	0.49	99.11	C <sub>25</sub> H <sub>42</sub> O <sub>12</sub>	3-Hydroxy-β-ionol 3-[glucosyl-(1 → 6)-glucoside]
22	7.16	547.2755	488.2617	0.93	98.43	C <sub>24</sub> H <sub>40</sub> O <sub>10</sub>	α-Ionol O-[arabinosyl-(1 → 6)-glucoside]
23	7.31	581.1877	582.1949	-0.11	98.91	C <sub>27</sub> H <sub>34</sub> O <sub>14</sub>	10-Acetoxyligustroside
24	7.34	461.2391	462.2464	0.27	97.26	C <sub>22</sub> H <sub>38</sub> O <sub>10</sub>	Neryl rhamnosyl-glucoside
25	7.36	335.0772	336.0845	0.07	84.73	C <sub>16</sub> H <sub>16</sub> O <sub>8</sub>	5-O-Caffeoylshikimic acid
26	7.41	367.1035	368.1108	-0.13	98.78	C <sub>17</sub> H <sub>20</sub> O <sub>9</sub>	3-O-Caffeoyl-4-O-methylquinic acid
27	7.46	177.0195	178.0268	-0.93	87.22	C <sub>9</sub> H <sub>6</sub> O <sub>4</sub>	Esculetin
28	7.565	435.1294	436.1367	0.56	99.14	C <sub>21</sub> H <sub>24</sub> O <sub>10</sub>	Phloridzin
29	7.566	609.1492	610.1565	-5.06	99.06	C <sub>27</sub> H <sub>30</sub> O <sub>16</sub>	Rutin
30	7.69	917.2353	918.2425	0.57	98.57	C <sub>42</sub> H <sub>46</sub> O <sub>23</sub>	Kaempferol 3-(2-p-coumaroylsophoroside) 7-glucoside
31	7.74	225.113	226.1204	0.61	99.6	C <sub>12</sub> H <sub>18</sub> O <sub>4</sub>	Tuberonic acid
32	7.81	547.2397	502.2415	-0.11	99.4	C <sub>24</sub> H <sub>38</sub> O <sub>11</sub>	3-Oxo-α-ionol 9-[apiosyl-(1 → 6)-glucoside]
33	7.93	593.1524	594.1592	-1.28	96.82	C <sub>27</sub> H <sub>30</sub> O <sub>15</sub>	Vitexin 4'-O-galactoside
34	7.94	901.2401	902.2472	0.93	99.02	C <sub>42</sub> H <sub>46</sub> O <sub>22</sub>	Kaempferol 3-[2''-(6'''-coumaroylglucosyl)-rhamnoside] 7-glucoside
35	8.16	549.255	504.2567	0.8	96.8	C <sub>24</sub> H <sub>40</sub> O <sub>11</sub>	Blumenol C O-[apiosyl-(1 → 6)-glucoside]
36	8.26	755.1821	756.1895	0.85	98.95	C <sub>36</sub> H <sub>36</sub> O <sub>18</sub>	2''-(6''-p-Coumaroylglucosyl)quercitrin
37	8.46	353.088	354.0951	-0.02	98.57	C <sub>16</sub> H <sub>18</sub> O <sub>9</sub>	5Z-Caffeoylquinic acid
38	8.645	303.051	304.0582	0.28	98.56	C <sub>15</sub> H <sub>12</sub> O <sub>7</sub>	(±)-Taxifolin
39	8.74	623.1612	624.1678	2.01	81.21	C <sub>28</sub> H <sub>32</sub> O <sub>16</sub>	Isoscoparin 2''-O-glucoside
40	8.77	161.0244	162.0317	-0.06	86.95	C <sub>9</sub> H <sub>6</sub> O <sub>3</sub>	7-Hydroxycoumarin
41	8.846	457.2438	458.2512	0.75	84.52	C <sub>23</sub> H <sub>38</sub> O <sub>9</sub>	[6]-Gingerdiol 5-O-β-D-glucopyranoside
42	9.14	447.2232	448.2305	0.75	99.31	C <sub>21</sub> H <sub>36</sub> O <sub>10</sub>	Kenposide B
43	9.22	317.0666	318.0739	0.24	98.7	C <sub>16</sub> H <sub>14</sub> O <sub>7</sub>	Demethylsulochrin
44	9.34	497.1086	498.1159	0.55	99.1	C <sub>25</sub> H <sub>22</sub> O <sub>11</sub>	3,4-Dicaffeoyl-1,5-quinolactone
45	9.44	259.0612	260.0684	0.17	99.36	C <sub>14</sub> H <sub>12</sub> O <sub>5</sub>	Khellin
46	9.474	125.0246	126.0318	-1.08	87.6	C <sub>6</sub> H <sub>6</sub> O <sub>3</sub>	(Z)-Tamarindial
47	9.474	287.0575	288.0646	-4.16	91.94	C <sub>15</sub> H <sub>12</sub> O <sub>6</sub>	3',4',5,7-Tetrahydroxyisoflavanone
48	9.54	315.0874	316.0947	0.1	85.11	C <sub>17</sub> H <sub>16</sub> O <sub>6</sub>	7-O-Methylpeltogynol
49	9.574	577.2863	518.2724	0.6	97.43	C <sub>25</sub> H <sub>42</sub> O <sub>11</sub>	Blumenol C O-[rhamnosyl-(1 → 6)-glucoside]
50	9.963	135.0451	136.0523	0.61	99.81	C <sub>8</sub> H <sub>8</sub> O <sub>2</sub>	3-(3-Furanyl)-2-methyl-2-propenal
51	10.0	287.0565	288.0638	-1.37	98.74	C <sub>15</sub> H <sub>12</sub> O <sub>6</sub>	Eriodictyol
52	10.252	345.0979	346.1052	0.22	97.68	C <sub>18</sub> H <sub>18</sub> O <sub>7</sub>	2,8-Dihydroxy-3,9,10-trimethoxypterocarpan
53	10.29	327.2178	328.225	-0.23	84.66	C <sub>18</sub> H <sub>32</sub> O <sub>5</sub>	9,12,13-Trihydroxy-10,15-octadecadienoic acid
54	10.528	289.0719	290.0792	-0.53	99.56	C <sub>15</sub> H <sub>14</sub> O <sub>6</sub>	(±)-Catechin
55	10.553	635.1405	636.1478	0.2	98.98	C <sub>32</sub> H <sub>28</sub> O <sub>14</sub>	Kaempferol 3-(3''-acetyl-6''-p-coumaroylglucoside)
56	10.629	207.0664	208.0737	-0.65	98.27	C <sub>11</sub> H <sub>12</sub> O <sub>4</sub>	2,5-Dimethoxycinnamic acid
57	10.729	301.0719	302.0792	-0.59	98.4	C <sub>16</sub> H <sub>14</sub> O <sub>6</sub>	Hesperetin
58	10.817	329.2335	330.2407	-0.37	98.14	C <sub>18</sub> H <sub>34</sub> O <sub>5</sub>	11,12,13-Trihydroxy-9-octadecenoic acid
59	10.83	269.0458	270.053	-0.83	97.39	C <sub>15</sub> H <sub>10</sub> O <sub>5</sub>	Apigenin
60	10.93	271.0634	272.0707	-0.17	99.42	C <sub>15</sub> H <sub>12</sub> O <sub>5</sub>	(±)-Naringenin

(continued on next page)

**Table 2** (continued)

S/ N	RT (min)	Precursor ( <i>m/z</i> )	Accurate Mass	Diff (ppm)	Score (DB)	Formula	Tentative ID
61	11.081	285.0426	286.0498	-0.34	98.97	C <sub>15</sub> H <sub>10</sub> O <sub>6</sub>	Luteolin
62	11.181	327.2176	328.2249	0.29	98.78	C <sub>18</sub> H <sub>32</sub> O <sub>5</sub>	9,12,13-Trihydroxy-10,15-octadecadienoic acid (isomer)
63	11.381	283.0611	284.0683	0.73	99.19	C <sub>16</sub> H <sub>12</sub> O <sub>5</sub>	Biochanin A
64	11.557	315.051	316.0584	-0.28	98.77	C <sub>16</sub> H <sub>12</sub> O <sub>7</sub>	Isorhamnetin
65	11.909	603.1511	544.1371	-0.33	99.21	C <sub>30</sub> H <sub>24</sub> O <sub>10</sub>	(2 <i>S</i> ,2' <i>'S</i> ,3 <i>S</i> ,3' <i>'R</i> ,4 <i>S</i> )-3,4',5,7-Tetrahydroxyflavan(2 → 7,4 → 8)-3,4',5,7-tetrahydroxyflavan
66	12.122	165.0193	166.0267	-0.3	99.32	C <sub>8</sub> H <sub>6</sub> O <sub>4</sub>	3,4-Methylenedioxybenzoic acid
67	12.511	359.0773	360.0846	-0.1	98.97	C <sub>18</sub> H <sub>16</sub> O <sub>8</sub>	Chrysosplenol F
68	12.599	345.0981	346.1053	-0.05	99.2	C <sub>18</sub> H <sub>18</sub> O <sub>7</sub>	2,8-Dihydroxy-3,9,10-trimethoxyterocarpan isomer
69	12.763	329.0666	330.0743	-0.92	98.69	C <sub>17</sub> H <sub>14</sub> O <sub>7</sub>	7,3',4'-Trihydroxy-3,8-dimethoxyflavone
70	13.001	283.0625	284.0696	-3.84	98.72	C <sub>16</sub> H <sub>12</sub> O <sub>5</sub>	1,5-Dihydroxy-2-methoxy-6-methylanthraquinone
71	13.516	329.0672	330.0744	-1.43	98.35	C <sub>17</sub> H <sub>14</sub> O <sub>7</sub>	7,3',4'-Trihydroxy-3,8-dimethoxyflavone
72	13.792	299.0562	300.0636	-0.63	97.95	C <sub>16</sub> H <sub>12</sub> O <sub>6</sub>	Diosmetin
73	13.943	627.3537	568.3403	-0.59	88.6	C <sub>34</sub> H <sub>48</sub> O <sub>7</sub>	(24 <i>E</i> )-3 $\alpha$ ,15 $\alpha$ -Diacetoxy-23-oxo-7,9(11),24-lanostatrien-26-oic acid
74	14.47	579.3688	534.3707	0.4	99.33	C <sub>35</sub> H <sub>50</sub> O <sub>4</sub>	Pyrohyperforin
75	14.733	611.3599	612.367	-1.3	98.3	C <sub>36</sub> H <sub>52</sub> O <sub>8</sub>	(24 <i>E</i> )-3 $\beta$ ,15 $\alpha$ ,22 <i>S</i> -Triacetoxylanosta-7,9(11),24-trien-26-oic acid
76	14.896	243.1756	244.1829	-0.72	84.52	C <sub>17</sub> H <sub>24</sub> O	Falcarinol
77	15.75	593.3487	548.3513	0.37	72.24	C <sub>36</sub> H <sub>44</sub> N <sub>4</sub> O	Manzamine A
78	16.089	593.3483	594.3556	0.07	73.97	C <sub>36</sub> H <sub>50</sub> O <sub>7</sub>	Glycosyl-4,4'-diaponeurosporenoate
79	16.302	291.1965	292.2037	0.33	85.61	C <sub>18</sub> H <sub>28</sub> O <sub>3</sub>	13- <i>epi</i> -12-oxo Phytodienoic Acid
80	16.804	647.3801	648.3873	0.1	99.61	C <sub>36</sub> H <sub>56</sub> O <sub>10</sub>	2 $\alpha$ -Hydroxygypsogenin 3- <i>O</i> - $\beta$ -D-glucoside
81	16.867	575.3379	530.3413	-3.27	58.43	C <sub>35</sub> H <sub>46</sub> O <sub>4</sub>	(-)-Neolinderatin
82	17.608	881.5201	836.5231	-0.47	69.12	C <sub>53</sub> H <sub>72</sub> O <sub>8</sub>	Amitenone
83	17.96	263.2014	264.2087	0.72	99.24	C <sub>17</sub> H <sub>28</sub> O <sub>2</sub>	10 <i>E</i> -Heptadecen-8-ynoic acid
84	20.306	271.228	272.2353	-0.4	99.4	C <sub>16</sub> H <sub>32</sub> O <sub>3</sub>	16-Hydroxyhexadecanoic acid
85	20.42	441.3587	382.3448	-0.19	99.73	C <sub>24</sub> H <sub>46</sub> O <sub>3</sub>	3-Oxo-tetracosanoic acid

Rt: Retention time in minutes.

16.089 min), monoterpene, pyrohyperforin (Rt 14.47 min), diterpene, amitenone (Rt 17.608 min) and triterpene saponin, 2 $\alpha$ -hydroxygypsogenin 3-*O*- $\beta$ -D-glucoside (Rt 16.804 min). Many of these terpene compounds are being reported in *C. odorata* aerial parts for the first time and therefore requires further characterization with other spectral techniques to confirm their presence. The group of compounds tentatively identified places terpenes and terpenoid derivatives as another major class of specialized metabolites present in *C. odorata*.

### 3.1.3. Phenolic compounds

Phenolic compounds constitute one of the predominant groups of specialized metabolites in plants and continue to engender strong research interest due to the important roles they play in both plant and animal protection. Natural phenolic compounds have been associated with numerous pharmacological and health benefits including antioxidant, anti-inflammatory, immunomodulatory, antimicrobial, neuroprotective, and anticancer properties. The genus, *Chromolaena*, is noted for the presence of diverse phenolic compounds represented in phenolic acids, flavonoids as well as numerous derivatives.

### 3.1.4. Simple phenolics and derivatives

Free phenolic acids in the form of hydroxybenzoic acid, hydroxycinnamic acid, as well as their derivatives were the most prominent simple phenolic compounds in *C. odorata* lipophilic extract. Compound #4 (Rt 5.256 min) furnished a

precursor ion at *m/z* 169.0141 and was assigned as gallic acid (Yunle Huang et al., 2022), a hydroxybenzoic acid derivative. The MS/MS fragment ion at *m/z* 125.0243 (Fig. S2) was deemed to arise from the characteristic neutral-loss of CO<sub>2</sub> from the precursor ion [M-H-44]<sup>-</sup> (Khallouki et al., 2015; Newsome et al., 2016). Gallic acid is one of the most widely distributed and studied plant phenolic compounds. Gallic acid is known to protect biomolecules from oxidative damage by scavenging of reactive chemical species. It is also reported to exert antifungal, antimicrobial, antioxidant, and anticancer activities (Sagdicoglu Celep et al., 2022). Meanwhile, the compound eluted at Rt 6.336 min was putatively identified on the basis of its precursor ion (*m/z* 153.0193) as 3,4-dihydroxybenzoic acid, which is often referred to as protocatechuic acid (Kumar et al., 2017). The putative identity of the compound was confirmed on the basis of the spectral data and fragment ion at *m/z* 136.9368, which corresponds to dihydroxybenzoic acid with lost hydroxyl group [M-H-17]<sup>-</sup>. Literature search also confirmed the presence of high levels of 3,4-dihydroxybenzoic acid in *C. odorata* aerial parts extracted with diethyl ether (Phan et al., 2001). Hydroxycinnamic acids are another group of phenolic acids commonly found in plant materials. According to Chaowuttikul et al., *C. odorata* leaves contain large amounts of chlorogenic acids, albeit the identities of the individual metabolites were not enunciated (Chaowuttikul et al., 2020). Herein, several hydroxycinnamates were identified in COLE. QTOF-MS data revealed a characteristic fragment ion at *m/z* 191.0559 for compounds number #9, #26, and #37. This fragment (also present as com-



pound #2) is likely derived from deprotonated quinic acid [M-H]<sup>-</sup> moiety (Kumar et al., 2017). Considering the molecular ion at  $m/z$  353.0877 as well as the quinic acid fragment ( $m/z$  191), which is likely due to the liberation of a caffeoyl moiety [M-H-162]<sup>-</sup>, compound #9 was tentatively assigned as chlorogenic acid (Long et al., 2012). Similarly, compound #26 was ascribed to 3-*O*-caffeoyl-4-methylquinic acid because of the parent ion being featured at  $m/z$  367.1035 and base peak at  $m/z$  191.0559 corresponding to the release of caffeoyl and methyl moieties from the molecular ion [M-H-162-14]<sup>-</sup>. The identity of compound #37 was established as 5-*Z*-caffeoylquinic acid, a chlorogenic acid isomer based on database search of the parent ion  $m/z$  353.088 and MS2 fragments. Besides the aforementioned quinic acid derivatives, a coumaric acid derivative with  $m/z$  497.1086 [M-H]<sup>-</sup> eluted at Rt 9.348 min was identified as 3,4-dicafeoyl-1,5-quinolactone.

### 3.1.5. Flavonoid derivatives

Several flavonoids were putatively identified in *C. odorata* lipophilic extract. These included many derivatives of quercetin and kaempferol. For example, compound #15 was putatively identified as quercetin 3-glucosyl-(1 → 2)-[rhamnosyl-(1 → 6)-galactoside] while compound #29 was established as rutin. The flavonoid presented a parent ion at  $m/z$  609.1492 and daughter ion at 300.0273, corresponding to the deprotonated aglycone ([Y-H]<sup>-</sup>) following cleavage of the rutinose moiety [M-H-308]<sup>-</sup> from the parent ion (Kumar et al., 2017). The QTOF MS data for compound #36 (Rt 8.268 min) featured a precursor ion at  $m/z$  755.1821 and a product ion at  $m/z$  609.1447, likely due to the loss of a coumaroyl moiety [M-H-146]<sup>-</sup> leaving behind a glucosylquercitrin fragment. With reference to the deprotonated molecular ion peak at  $m/z$  755.1822, compound #36 was assigned as 2''-(6''-*p*-coumaroylglucosyl)quercitrin whereas compound #64 with pseudomolecular peak at  $m/z$  273.0769 was assigned as the flavonoid aglycone isorhamnetin (Im et al., 2018). Regarding compound #28 (Rt 7.565 min), a deprotonated molecular ion was observed at  $m/z$  435.1294. The MS/MS data featured a prominent product ion at  $m/z$  273.0763 caused by the loss of 162 amu. By matching the MS data with database as well as comparing with previous literature reports, this compound was identified as phloridzin or phloretin-2-glucoside (Prakash et al., 2019). Additionally, several kaempferol derivatives were putatively identified including compound #18, kaempferol 3-sophoroside 7-rhamnoside (Rt 7.01 min), compound #30 kaempferol 3-(2-*p*-coumaroylsophoroside) 7-glucoside (Rt 7.69 min), compound #34 kaempferol 3-[2''-(6''-coumaroylglucosyl)-rhamnoside] 7-glucoside (Rt 7.94 min), and compound #55 kaempferol 3-(3''-acetyl-6''-*p*-coumaroylglucoside) (Rt 10.55 min). For compound #54, a deprotonated parent ion peak at  $m/z$  289.0719 was observed and the compound was assigned as (±)-catechin. MS/MS data yielded a characteristic product peak at  $m/z$  139 which likely originated from ring A as a result of cleavage of two bonds in ring C (i.e., O-C of position 1 and 2, and C-C of position 3 and 4) of the molecule (Spáčil et al., 2010). Besides, compound #72 (Rt 13.792 min) was found as the methoxylated flavonoid aglycone, diosmetin which yielded pseudomolecular ion at  $m/z$  299.0562 and a strong fragment peak at  $m/z$  284.0767. The fragment at  $m/z$  284 is thought to arise due to loss of CH<sub>3</sub> from the deprotonated molecular ion [M-H-CH<sub>3</sub>]<sup>-</sup> (Justesen, 2000). Com-

pound #59 presented a deprotonated ion at  $m/z$  269.0458, with prominent MS/MS fragments at  $m/z$  151.0034 and  $m/z$  117.0346. On the basis of previously reported literature and database search, the compound was identified as apigenin (Sánchez-Rabáneda et al., 2004). Other flavonoid aglycones, many of which had been previously found in *C. odorata* were also identified from the QTOF-MS data of COLE. These included phloridzin (Rt 7.565 min), (±)-taxifolin (Rt 8.645), eriodictyol (Rt 10.026 min), hesperetin (Rt 10.729), apigenin (Rt 10.83 min), (±)-naringenin (10.93 min) and luteolin (Rt 11.081 min), as well as biochanin A (Rt 11.381 min) and (-)-neolinderatin (Rt 16.867 min) (Yuan et al., 2007).

### 3.1.6. Other compounds

Fatty acids and fatty acyl glycosides were noticed as another important group of compounds based on the number of constituents identified in the aerial part of *C. odorata* lipophilic extract. Seven fatty acyl glycosides were tentatively identified in COLE, including (*Z*)-2-methyl-2-butene-1,4-diol 4-*O*-α-D-glucopyranoside (Rt 5.934), 3-*O*-(α-L-rhamnopyranosyl-(1 → 2)-α-L-rhamnopyranosyl)-3-hydroxydecanoic acid (Rt 5.959), 5α,6α-Epoxy-7*E*-megastigmene-3β,9ξ-diol 9-glucoside (Rt 7.038 min), α-Ionol *O*-[arabinosyl-(1 → 6)-glucoside] (Rt 7.164 min), 3-oxo-α-ionol 9-[apiosyl-(1 → 6)-glucoside] (Rt 7.817 min), and blumenol C *O*-[apiosyl-(1 → 6)-glucoside] (Rt 8.168 min). In all, a total of 85 secondary metabolites were putatively identified in COLE by UPLC-MS.

### 3.1.7. GC-MS analysis of *C. odorata* lipophilic extract

A number of previous reports have highlighted the beneficial roles of volatile and semi-volatile constituents from *C. odorata*. Most of these reports however focused on the essential oil as the source of volatile compounds. COLE presents an opportunity to further extend the coverage of specialized metabolites in *C. odorata* aerial parts. The volatile and semi-volatile constituents of COLE were profiled by GC-MS analysis. A total of thirty six compounds were tentatively identified. These volatile metabolites can be broadly categorized into hydrocarbons, sesquiterpene and derivatives, terpenoids, fatty acids and derivatives, as well as flavonoids (Table 3). The major composition of volatile compounds were found to be sesquiterpene and their derivatives, which made up 55.38 % of the total.

Besides, COLE also presented the following compounds with considerably high amounts, viz; γ-eudesmol (1.42 %), β-eudesmol (4.38 %), α-eudesmol (5.18 %), lupeol (4.9 %), (+)-δ-cadinene (8.75 %), (-)-β-caryophyllene (9.15 %). Interestingly, some of these compounds have been reported to demonstrate desirable biological and pharmacological properties. For instance, the oxygenated sesquiterpenoid compound, β-eudesmol was previously found to stimulate appetite (Ohara et al., 2017), inhibit glucosyltransferase and reduce dental caries (Shruthi et al., 2021), demonstrate antiangiogenic (Tsuneki et al., 2005), and antitumor properties (Ma et al., 2008). Similarly, δ-cadinene reportedly suppressed the growth of ovarian cancer cells through caspase-dependent apoptosis and cell cycle arrest (Hui et al., 2015), whereas the triterpenoid, lupeol was reported to exhibit potent anti-inflammatory and anticancer activities (Liu et al., 2021). Considering that β-caryophyllene constituted almost one-tenth of the total volatiles in COLE, it is only germane to mention that the sesquiterpene has been reported to possess antioxidant, antimicrobial,

**Table 3** GC–MS qualitative analysis of compounds present in lipophilic extract of *C. odorata* aerial parts.

S/ N	RT (min)	Name (Tentative ID)	Formula	CAS RN	% of Total	Nature of Compound
1	11.4127	Undecane	C <sub>11</sub> H <sub>24</sub>	1120–21-4	1.23	Hydrocarbon
2	12.5354	Geijerene	C <sub>12</sub> H <sub>18</sub>	6902–73-4	1.15	Hydrocarbon
3	14.2355	Tridecane	C <sub>13</sub> H <sub>28</sub>	629–50-5	0.22	Hydrocarbon
4	17.9179	Bicycloelemene	C <sub>15</sub> H <sub>24</sub>	2000216–46-8	0.27	Elemene sesquiterpenoids
5	18.9188	α-Copaene	C <sub>15</sub> H <sub>24</sub>	3856–25-5	1.94	Hydrocarbon
6	20.0158	(-)-β-Caryophyllene	C <sub>15</sub> H <sub>24</sub>	87–44-5	9.15	Bicyclic sesquiterpene
7	20.2532	β-Cubebene	C <sub>15</sub> H <sub>24</sub>	13744–15-5	3.51	Tricyclic sesquiterpene
8	20.3815	γ-Elemene	C <sub>15</sub> H <sub>24</sub>	29873–99-2	0.37	Sesquiterpene
9	20.869	α-Humulene	C <sub>15</sub> H <sub>24</sub>	6753–98-6	2.72	Monocyclic sesquiterpene
10	21.4464	γ-Muuroleone	C <sub>15</sub> H <sub>24</sub>	30021–74-0	1.61	Sesquiterpene
11	21.5491	Germacrene D	C <sub>15</sub> H <sub>24</sub>	23986–74-5	3.38	Sesquiterpene
12	21.671	Aromadendrene	C <sub>15</sub> H <sub>24</sub>	489–39-4	0.58	5,10-Cycloaromadendrane sesquiterpenoids
13	21.8121	cis-Muurolo-4(15),5-diene	C <sub>15</sub> H <sub>24</sub>	157477–72-0	0.38	Carbobicyclic compound
14	21.8891	Cubebol	C <sub>15</sub> H <sub>26</sub> O	23445–02-5	2.14	Sesquiterpene alcohol
15	22.0302	α-Muuroleone	C <sub>15</sub> H <sub>24</sub>	31983–22-9	1.11	Sesquiterpene
16	22.5755	(+)-δ-Cadinene	C <sub>15</sub> H <sub>24</sub>	483–76-1	8.75	Sesquiterpene
17	23.0374	α-Calacorene	C <sub>13</sub> H <sub>16</sub>	30364–38-6	0.09	Sesquiterpene
18	23.185	α-Elemol	C <sub>15</sub> H <sub>26</sub> O	639–99-6	1.43	Sesquiterpene
19	23.5827	τ-Cadinol	C <sub>15</sub> H <sub>26</sub> O	11/1/5937	1.76	Sesquiterpene
20	23.8329	(-)-Spathulenol	C <sub>15</sub> H <sub>24</sub> O	77171–55-2	1.64	Tricyclic sesquiterpene
21	23.9613	Caryophyllene oxide	C <sub>15</sub> H <sub>24</sub> O	1139–30-6	3.2	Bicyclic sesquiterpene
22	24.1601	Viridiflorol	C <sub>15</sub> H <sub>26</sub> O	552–02-3	0.6	Sesquiterpene
23	24.5643	(-)-Humulene epoxide II	C <sub>15</sub> H <sub>24</sub> O	19888–34-7	0.55	Sesquiterpenoid
24	25.0134	τ-Cadinol acetate	C <sub>17</sub> H <sub>28</sub> O <sub>2</sub>	149197–48-8	0.95	Sesquiterpene
25	25.0711	(+)-γ-Eudesmol	C <sub>15</sub> H <sub>26</sub> O	1209–71-8	1.42	Oxygenated sesquiterpene
26	25.4753	β-Eudesmol	C <sub>15</sub> H <sub>26</sub> O	473–15-4	4.38	Oxygenated sesquiterpene
27	25.5523	α-Eudesmol	C <sub>15</sub> H <sub>26</sub> O	473–16-5	5.18	Oxygenated sesquiterpene
28	28.946	Cryptomeridiol	C <sub>15</sub> H <sub>28</sub> O <sub>2</sub>	2000334–99-5	0.59	Sesquiterpene alcohol
29	31.2556	Methyl palmitate	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	112–39-0	0.68	Fatty acid methyl ester
30	32.5643	Ethyl palmitate	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	628–97-7	2.92	Fatty acid ethyl ester
31	34.7199	Phytol	C <sub>20</sub> H <sub>40</sub> O	150–86-7	2.71	Hydrogenated diterpene alcohol
32	35.0022	Methyl isostearate	C <sub>19</sub> H <sub>38</sub> O <sub>2</sub>	5129–61-3	0.33	Fatty acid methyl ester
33	35.5988	Methyl linolelaidate	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	2566–97-4	1.06	Fatty acid methyl ester
34	35.7143	Ethyl Linoleate	C <sub>20</sub> H <sub>36</sub> O <sub>2</sub>	544–35-4	2.28	Fatty acid ethyl ester
35	49.8923	2'-Hydroxy-4,4',5',6'-tetramethoxychalcone	C <sub>19</sub> H <sub>20</sub> O <sub>6</sub>	41929–26-4	1.8	Flavanone
36	56.8017	Lupeol	C <sub>30</sub> H <sub>50</sub> O	545–47-1	4.9	Pentacyclic terpenoid

Rt: retention time; CAS RN: CAS Registry Number.

anticarcinogenic and anticancer potentiating effects (Legault and Pichette, 2007). The presence of these specialized metabolites in COLE in significant amounts is a strong pointer that it could be effectively valorized for biomedical and pharmacological purposes.

### 3.2. *C. odorata* lipophilic extract demonstrated potent anticancer activities

The cytotoxic activity of COLE against human breast cancer (MCF-7 and MDA-MB-231), colon cancer (HT-29 and HCT-116), as well as non-cancer (HEK-293 and CCD-841 CoN) cells was investigated using MTT assay. Cells were exposed to various concentrations of COLE and after 72 h, the cytotoxic activity was determined. The results of the cytotoxic effect of COLE are summarized in Table 4 and Fig. 3. It

can be seen that COLE exhibited potent cytotoxic effect against the cancer cell lines with IC<sub>50</sub> in the range of 9.18 – 19.48 µg/mL. Also, it can be observed that COLE was more active against the breast cancer cells than the colon cancer cells, and the most potent effect was noticed against MCF-7 cells. Regarding the cytotoxic effect of COLE against normal cell lines, COLE was found to induce some cytotoxic effect. But when compared to the effect of COLE against cancer cells, the cytotoxic effect against normal cells were significantly lower. In fact, according to the recommendations of the US National Cancer Institute for *in vitro* screening of anti-cancer agents, botanicals such as plant extract are expected to display IC<sub>50</sub> values below 20 µg/mL to be considered cytotoxic (Geran et al., 1972).

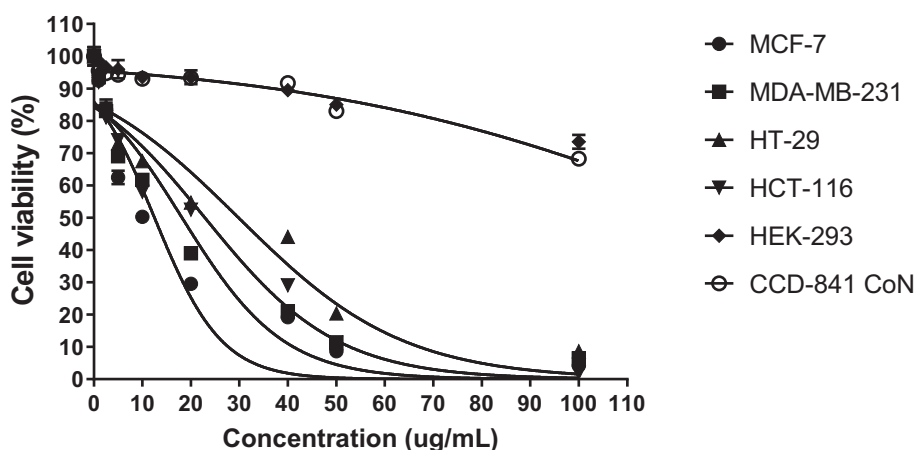
In this context, the obtained large IC<sub>50</sub> values of COLE against normal cells (≥198 µg/mL) suggests that the extract

**Table 4** *In vitro* Cytotoxic Activities of COLE<sup>a</sup>.

Cell line	Classification	COLE (IC <sub>50</sub> µg/mL)	COLE Selectivity Index <sup>b</sup>
MCF-7	Breast cancer cell	9.18 ± 0.21	21.59 – 41.86
MDA-MB-231	Breast cancer cell	12.19 ± 0.43	16.26 – 31.53
HT-29	Colon cancer cell	19.48 ± 0.11	10.17 – 19.73
HCT-116	Colon cancer cell	14.12 ± 0.50	14.04 – 27.22
HEK-293	Kidney normal cell	198.2 ± 2.89	–
CCD-841 CON	Colon normal cell	384.3 ± 4.14	–

<sup>a</sup> The treatment period was 72 h. Data represent the mean ± standard deviation of triplicate.

<sup>b</sup> The SI was obtained as the average of the IC<sub>50</sub> value of the normal cell line divided by the IC<sub>50</sub> value of the cancer cell line obtained in each independent experiment.



**Fig. 3** Impact of various concentrations of *C. odorata* lipophilic extract on viability of cells after 72 h determined by MTT assay.

was not cytotoxic on these cells. At the same time, it must be emphasized that while it is important for prospective anticancer extracts to display potent effects against cancer cells, such cytotoxic effects should not be extended against normal healthy cells. At most, the cytotoxic effect against normal healthy cells should be minimal. For *in vitro* anti-cancer drug screening, the relative effect of anticancer agents against normal cells vis-à-vis cancer cells can be estimated using the selectivity index (SI). Anticancer agents with high SI values ( $\geq 10$ ) tend to be more selective towards cancer cells and less active against normal cells (Peña-Morán et al., 2016). As shown in Table 4, COLE displayed substantially high SI values in the range of 10.17 – 41.86, indicating that the anticancer effect of the extract is likely to be restricted only to the cancerous cells.

Previous studies on the anticancer effects of *C. odorata* extracts revealed some notable findings. Yusuf and co-workers recently reported that crude ethanol extract from *C. odorata* leaves inhibited the proliferation of HepG2 cancer cells and induced cell cycle arrest. The authors also found that following 24 h period of cell exposure to the samples, the crude extract was more potent with IC<sub>50</sub> of 23.44 µg/mL compared to the hexane (84.54 µg/mL), ethyl acetate (167.49 µg/mL) and ethanol (88.59 µg/mL) fractions. Meanwhile, both the extract and fractions were less cytotoxic to normal Vero cells with IC<sub>50</sub> > 950 µg/mL (Yusuf et al., 2022b). In the same vein, while evaluating the anticancer effect of *C. odorata*, Singh

noticed that the crude ethanol extract demonstrated modest cytotoxic effects against HCT116 (IC<sub>50</sub> 60.18 µg/mL) and HeLa cells (IC<sub>50</sub> 71.74 µg/mL) (Singh, 2012). When crude ethanol extract was fractionated, Yusuf et al. found that the soluble ethyl acetate fraction was able to inhibit the proliferation of HeLa cervical cancer cells after 24 h with an IC<sub>50</sub> value of 82.41 µg/mL (Yusuf et al., 2022a). Two points are worth noting from these earlier studies. Firstly, they underscore the anticancer potential of *C. odorata* extract. Secondly, although not clearly stated, the authors implicitly identified the need to improve the anticancer property of the crude extract. This was accomplished via further fractionation using organic solvents. However, success of the fractionation process, that is, collection of the active fraction from the crude extract is not guaranteed. In other cases, the collection of the active fraction may involve the use of toxic organic solvents which poses serious health and environmental concerns and complicates the downstream formulation processes (Singh, 2012; Yusuf et al., 2022a). Although we agree with the need to target the preparation of extracts with high anticancer activity, we also bore in mind the necessity to reduce/eliminate potentially noxious organic solvents during the preparation process; thus, the method adopted. Compared to the afore-mentioned previous studies, COLE displayed superior anticancer activity *in vitro* and acceptable selectivity. The presence and prominence of specialized metabolites with known anticancer attributes, including,  $\beta$ -caryophyllene and caryophyllene oxide, (Fidy

et al., 2016; Lei et al., 2021),  $\delta$ -cadinene (Hui et al., 2015),  $\beta$ -eudesmol (Ma et al., 2008), and lupeol (Liu et al., 2021) in the lipophilic extract further supports the anticancer properties of COLE. For instance,  $\beta$ -caryophyllene was reported to affect the growth and proliferation of many cancer cells. In non-small cell lung cancer cells, treatment with the natural sesquiterpene was reported to enhance apoptotic rate, inhibit growth, elevate the level of antioxidant enzymes, miR-659-3p, apoptotic factors, and reduce the level of oxidative stress and SphK1 (Lei et al., 2021). Similarly, according to Dahham et al., treatment of HCT-116 and HT-29 (colon cancer) cells, as well as PANC-1 (pancreatic cancer) cells with  $\beta$ -caryophyllene resulted in strong growth inhibition of the cancer cell lines (Dahham et al., 2015). Whereas it was revealed that when multiple myeloma cells were exposed to  $\beta$ -caryophyllene treatment, the sesquiterpene caused antiproliferative effects via the induction of apoptosis and modulation of cell cycle (Mannino et al., 2021). Moreover,  $\beta$ -caryophyllene oxide was previously found to inhibit the growth of human prostate cancer (PC-3) and breast cancer (MCF-7) cells. Treatment of the cancer cells with the compound reportedly induce ROS-mediated MAPKs activation, inhibit PI3K/AKT/mTOR/S6K1 signaling cascades, and induce apoptosis via suppression of gene products that mediate proliferation, angiogenesis, metastasis, and tumor cell survival.  $\alpha$ -Humulene, an isomer of  $\beta$ -caryophyllene was equally observed to exert anticancer activity. It was revealed that the sesquiterpene exhibited significant antiproliferative effect against CaCo-2 intestinal cancer cells (Ambrož et al., 2015). Beside,  $\beta$ -caryophyllene and  $\beta$ -caryophyllene oxide were also shown to synergistically potentiate the anticancer activity of other natural metabolites such as  $\alpha$ -humulene and the synthetic anticancer agent, doxorubicin (Park et al., 2011). Meanwhile,  $\beta$ -eudesmol, another compound present in COLE, was reported to inhibit superoxide production, proliferation, adhesion and migration of lung cancer (A549) and colon cancer (HT29) cells. The sesquiterpene was also found to inhibit tumor growth via the suppression of tumor neovascularization and tumor cell proliferation (Ben Sghaier et al., 2016; Ma et al., 2008). Similarly, it has been noted that lupeol suppressed the proliferation and migration of MDA-M-231 breast cancer cells through a crosstalk mechanism between EMT and autophagy (Zhang et al., 2022). As a phytocomplex harboring all these bioactive constituents, it is reasonable to deduce that the mode of COLE anticancer activity probably involves a number of the aforementioned mechanisms working together in synergy or addition. To the best of our knowledge, this is the first report on the anticancer properties of the lipophilic fraction of *C. odorata* aerial part extract prepared in an innocuous manner, and the findings highlight the potential therapeutic benefits of COLE in the management of cancer disease.

### 3.3. Antimicrobial activities of COLE

Plant extracts remain an indispensable and invaluable resource for the discovery and development of important antimicrobial formulations. In many parts of the world, formulations based on plant extracts are still very much relied upon for countering various forms of microbial infections. Pertaining to the antimicrobial activity of extracts from edible parts of plants, it has been postulated that highly active extracts exhibit MIC

values  $< 100 \mu\text{g/mL}$ , significantly active extracts feature  $100 \leq \text{MIC values} \leq 512 \mu\text{g/mL}$ , those with moderate activity present  $512 \leq \text{MIC values} \leq 2048 \mu\text{g/mL}$ , low activity if the MIC values  $> 2048 \mu\text{g/mL}$ , and inactive if the MIC values  $> 10 \text{ mg/mL}$  (Tamokou et al., 2017). The antimicrobial property of two different fractions prepared from *C. odorata* extract was evaluated via broth microdilution technique. The MIC and MBC values are presented in Table 5. From the MIC values, it is apparent that both fractions demonstrated antimicrobial activity, although the lipophilic fraction was comparatively more potent. The lipophilic fraction was significantly active against *B. cereus* (MIC value of 0.16 mg/mL) and moderately active against *E. coli* (MIC value of 1.06 mg/mL) and *L. monocytogenes* (MIC value of 0.63 mg/mL). Meanwhile, the hydrophilic extract presented low activity towards all the microbial pathogens. These findings were in accord with previous reports by Naidoo et al., who observed that the aqueous, but not methanolic or ethyl acetate extract did not inhibit *B. cereus* and the other microbes investigated (Naidoo et al., 2011). Similarly, Omokhua et al. noted that the non-polar extract of *C. odorata* was more enriched with antimicrobial agents compared to the polar extracts (Omokhua et al., 2017). Comparatively, the results also seemed to suggest that COLE exhibited better antimicrobial action against the gram-positive microbial strains relative to the gram negative microbial strain. This may be due to the presence of the outer membrane on the gram-negative bacteria which affords them an additional layer of protection against antibiotic agents. As presented in Table 2, COLE is endowed with several notable phenolic compounds, such as ( $\pm$ )-catechin, luteolin, isorhamnetin, gallic acid, chlorogenic acid, quinic acid, hesperetin, apigenin, naringenin, rutin, amongst others. These phenolic compounds have been reported to exert good antibacterial activities against many different bacteria, inclusive of those used in the current study (Adamczak et al., 2019; Fu et al., 2016). Similarly, terpenes and their derivatives such as  $\beta$ -caryophyllene, phytol, lupeol, (+)- $\delta$ -cadinene, germacrene D, and  $\beta$ -eudesmol, were present in large amounts in COLE as evidenced in the GC-MS results (Table 3). It is worthy to mention that these compounds, especially  $\beta$ -caryophyllene, the dominant volatile constituent, are also known to strongly inhibit the growth and/or cause the death of microbial pathogens such as fungi and bacteria (Dahham et al., 2015; Selestino Neta et al., 2017). Interestingly, it was previously reported that amongst the volatile constituents, those with hydroxyl groups (phenolic and alcohol), compared to the hydrocarbons, were far more efficacious (Guimarães et al., 2019). Thus, the antimicrobial activity of COLE can be attributed to the presence of not just a single compound, but rather the ensemble of different groups of compounds present in the extract including phenolics, flavonoids, terpenes, and terpenoids. Pertaining to the mode of antimicrobial action, it is probable that COLE exerts its antimicrobial effect via a multipronged manner which could be linked to those of phenolics, flavonoids and terpenes. This may involve binding to adhesins, damage to microbial cell wall, destabilization of cell membrane, as well as the permeation and interruption of vital intracellular functions through protein binding, enzyme inactivation, metal ions complexation, or induction of oxidative stress by constituents of the extract (Borges et al., 2013; Lee et al., 2016; Pérez Zamora et al., 2018). The good antibacterial activity of the lipophilic fraction of *C. odorata*, but not the hydrophobic fraction, sug-

**Table 5** Antimicrobial activity of *C. odorata* fractions.

Bacterial isolates	<i>C. odorata</i> lipophilic fraction		<i>C. odorata</i> hydrophilic fraction		Vancomycin/ Gentamicin <sup>a</sup>	
	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)
<i>B. cereus</i>	0.16	0.31	3.13	6.25	0.02 <	0.02 <
<i>E. coli</i>	1.06	2.01	6.25	12.5	0.02 <	0.02 <
<i>L. monocytogenes</i>	0.63	2.01	6.25	12.5	0.02 <	0.02 <

<sup>a</sup> Vancomycin was used as the positive control against *B. cereus* and *L. monocytogenes* whereas gentamicin was used against *E. coli*.

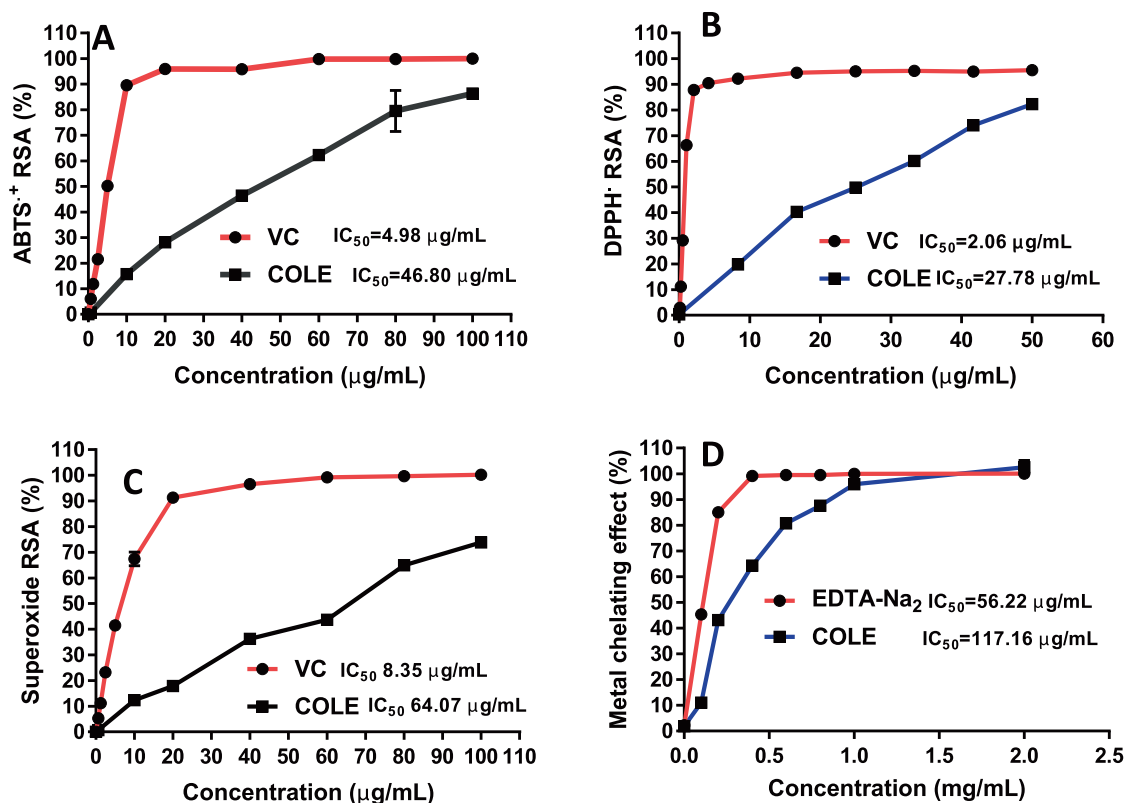
gest that it could be valorized toward the control of various pathogenic microbes. This could be of tremendous value as a cost-effective natural antibiotic in certain parts of the world with meagre economic means but abundant supply of *C. odorata*.

### 3.4. *C. odorata* lipophilic extract demonstrated potent antioxidant and metal chelating activities

There is ample evidence from many epidemiological studies suggesting an inverse relationship between the consumption of fruits and vegetable (reputable sources of dietary antioxidants) and reduced risk of cardiovascular, non-cardiovascular, cancer, and premature mortality (Aune et al., 2017; Liu et al., 2000). Consequently, studies involving plant extracts for food and nutraceutical applications routinely determine their antioxidant content. DPPH and ABTS assays

are well established *in vitro* techniques for evaluating the properties of compounds or extracts *in vitro*. While the ABTS assay measures the ability of the extract to donate electrons (SET: single electron transfer mechanism), the DPPH assay is based on the capacity of the analyte to donate electrons and hydrogen atoms to the radicals. Most natural product extracts employ more than a single radical scavenging mechanism to exert their antioxidant action and thus the need for multiple radical scavenging assays.

The *in vitro* antioxidant capacity of COLE was evaluated based on their ability to scavenge the cationic ABTS, anionic DPPH, and superoxide radicals. As shown in Fig. 4, COLE and ascorbic acid (the antioxidant standard) demonstrated potent radical scavenging properties and their effects were concentration dependent. The IC<sub>50</sub> value of COLE against ABTS, DPPH and superoxide radicals were 46.80 ± 3.11, 27.78 ± 0.98 and 64.07 ± 2.36 µg/mL. These values were



**Fig. 4** Antioxidant activity of COLE against (A) ABTS, (B) DPPH, (C) superoxide anion and (D) metal-ion chelating effect.

expectedly higher than those obtained for ascorbic acid (antioxidant standard) with  $IC_{50}$  values of  $4.98 \pm 0.32$ ,  $2.06 \pm 0.02$ ,  $8.35 \pm 0.43$   $\mu\text{g/mL}$  for inhibition of ABTS, DPPH and superoxide anion radicals, respectively, considering that the standard is a pure compound whereas COLE is a crude mixture of phytochemicals. However, it must be stated that when COLE was compared to literature reports of some common natural extracts with known antioxidant activities, such as guava leaves extract (Sampath Kumar et al., 2021), essential oil of guava leaves (Lee et al., 2012), polyphenol-rich extract from leaves (Nivedha et al., 2020), and *Eucalyptus camaldulensis* lipophilic extract (Yanping Huang et al., 2022), the  $IC_{50}$  values of COLE presented comparable, and in some cases superior antioxidant activity. The strong antioxidant effect of COLE can be attributed to the presence of multiple secondary metabolites with potent antioxidant properties, such as gallic acid, rutin, chlorogenic acid, ( $\pm$ )-catechin, hesperetin, and luteol.

Furthermore, metal ions such as iron and copper are known to be actively involved in free radical initiation (Duthie et al., 1997). Thus, it was worthwhile to determine the metal chelating activity of COLE. It was revealed that the lipophilic extract of *C. odorata* exhibited strong metal ion chelating capacity with an  $IC_{50}$  value of  $117.16 \pm 4.30$   $\mu\text{g/mL}$  (Fig. 4D) towards ferrous ions. This may be due to the high flavonoid content and presence of individual metabolites such as catechin, rutin, apigenin, and naringenin, known to be effective metal ion chelators (Cherrak et al., 2016; Jahanshahi et al., 2021; Spiegel and Sroka, 2023). Iron accumulation has been implicated in the development of degenerative diseases such as Parkinson's disease, Alzheimer's disease and cardiovascular disease. In cancers, excess iron has been tightly linked to tumorigenesis via a number of mechanisms. Excess iron may induce the generation of reactive oxygen species (ROS) via Fenton reaction. Elevated levels of ROS can cause severe damage to proteins, lipids, and DNA, consequently triggering tumorigenesis. Besides, the proliferating tumor cells require iron as an essential nutrient (Brown et al., 2020). Authors have documented in literature the close association between elevated levels of iron and development of multiple human cancer types, such as breast cancer, lung cancer, prostate cancer, pancreatic cancer, hepatocellular cancer, and colorectal cancer. Thus, multiple therapeutic approaches based on the deprivation of iron has been proposed (Islam et al., 2022; Zhang and Zhang, 2015). Taken together, the good iron chelation activity, strong antioxidant activity, and selective anticancer property suggest that COLE could have positive implications in the amelioration of these conditions.

#### 4. Conclusions

Herein, we report for the first time the chemical and biological characterization of *C. odorata* aerial part lipophilic extract (COLE) prepared via a facile green chemistry approach. COLE was successfully prepared without the use of any innocuous organic solvent and found to contain a wide range of bioactive metabolites. Also, it was revealed that COLE was enriched predominantly with valuable terpenes, terpenoids, and phenolic constituents, contributing to its remarkable biological and pharmacological properties. In particular, the lipophilic extract was found to exhibit potent and selective anticancer properties towards breast and colon cancer cell lines, whereas over the range of concentrations tested, the extract was non-toxic to normal cell lines. The results also indicated that COLE possessed significant activity against *B. cer-*

*eus* and moderate activity against *L. monocytogenes* and *E. coli*. Furthermore, the extract was found to exert impressive radical scavenging and metal chelating activities, highlighting its capacity to neutralize free radical and oxidizing species. With the current global challenge of increasing incidence of chronic diseases associated with oxidative stress and cancers, the antioxidative and anticancer effects of COLE is a good indication that the extract could be considered as a promising cost-effective and efficacious pharmacological resource for combating these conditions. Thus, to maximize the potential health benefits of COLE, further studies aimed at elucidating its potency, potential adverse effects, precise mechanistic underpinnings, bioavailability, amongst others, in animal models are highly encouraged.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.arabjc.2023.104834>.

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