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Phytochemical Constituents and Antioxidant Activities of *Melaleuca leucadendra*, *Convolvulus arvensis,* and *Wedelia calenlulacea* Leaves Methanolic Extracts.

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ABSTRACT:

Introduction: Phytochemicals are bioactive chemical compounds naturally produced by plants. These natural compounds, have antioxidant properties, cytotoxic and anticancer activities, can be promising for prevention and treatment of some tumors.

Aim: The present study was conducted to explore both the phytochemical constituents and antioxidant activity of M. leucadendra, C. arvensis, and *W. calenlulacea* leaves methanolic extracts.

Methods: The phytochemical constituents from the leaves methanolic extracts of these three plants were analyzed by HPLC, and GC-MS. The antioxidant capacity of these extracts was determined by modern *in vitro* assays.

Results: The phytochemical analysis by HPLC, and GC-MS revealed good similarities of the presence of constituents of the methanolic extracts of these plants. However, HPLC analysis showed the absence of some constituents in the extract of *W. calenlulacea* as they appeared in the other two plants. Methanol extract of *M. leucadendra* by GC-MS analysis revealed the highest antioxidant and total phenolic, flavonoid, and saponin content. The three plants leaves extracts contain terpenoids as the most abundant phytochemicals that exert antioxidants. Our results appear to indicate the strongest antioxidant activity of *M. leucadendra* extract compared to *C. arvensis* and *W. calenlulacea* and this indicates a strong capacity to scavenge free radicals.

Conclusion: Among the three, *M. leucadendra* exhibited the highest levels of total phenolic, flavonoid, and saponin content, correlating with its superior antioxidant activity across various in vitro assays. These findings suggest that *M. leucadendra* may serve as a promising natural source of antioxidants for pharmaceutical or nutraceutical applications.

Keywords: phytochemical constituents, methanolic extract, *M. leucadendra*, *C. arvensis*, *W. calendulacea*, antioxidant.

1. INTRODUCTION

Phytochemicals are a diverse group of natural bioactive compounds of plants, frequently used in chemoprevention and chemotherapeutic treatment (Koomson et al., 2018). Some of the significant phytochemicals are alkaloids, polyphenols, terpenoids, phytosterols, saponins, and tannins (Nyamai et al., 2016). These phytochemicals exhibit biological properties such as antimicrobial, antiallergic, antiviral effects and possess strong antioxidant activities (Kumar et al., 2023), and anti-inflammatory, and anticancer properties (Davidova et al., 2024). Phytochemical constituents can give excellent anti-disease properties (Harith et al., 2018). Flavonoids are polyphenolic compounds known for their anti-inflammatory, antioxidant, and anticancer properties. Alkaloids are used as antimicrobials and in pain management. Terpenoids have anticancer, anti-inflammatory, and antiviral effects (Jerdikis, 2024). Numerous studies on plant extracts or individual bioactive compounds derived from medicinal plants have demonstrated a promising antioxidant activity treating many diseases. (Smruthi et al., 2021; Yadav et al., 2024; Al Naqbi et al., 2025) The different bioactive compounds in these plants are used in traditional medicine for pain relief and treatment of multiple disorders (Manisha et al., 2025). For decades, *Melaleuca leucadendra* plants have been used in folk medicines in different civilizations, belonging to the family Myrtaceae. These plants are known as tea trees in Australia (Baily and Baily, 1976). While bark and leaves of *M. leucadendra* were used for the relief of cold and flu symptoms (Packer et al., 2012), for obesity, and for hyperlipidemia (Saifudin et al., 2016).

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Several reports exist on the chemical composition of the volatile oils of various *Melaleuca* species from Brazil, Egypt, India, and Thailand (Altman, 1988; Farag et al., 2004; Silva, et al., 2007; Padalia et al. 2015; Tanavata et al., 2022; Hegazi et al., 2022). Besides the importance of essential oil in Genus *Melaleuca*, the nonvolatile components in this plant are of great value for its anticancer, anti-inflammatory, antimicrobial, antioxidant, neuroprotective, and hepatoprotective, activities (Kanso et al., 2022). Leaves methanolic extract of the plant revealed the presence of saponins, terpenoids, alkaloids steroids, and tannins (Khongsai & Vittaya, 2019).

C. arvensis is a deep-rooted weed that belongs to the family Convolvulaceae. (Arora and Malhotra, 2011; Abdul Jalill et al., 2014). The leaves and roots are used as laxative and anti-hemorrhagic (Austin, 2000). The plant contains flavonoids, tannins, alkaloids, saponins, and polyphenolic compounds (Kaur & Kalia, 2012a). It has antioxidant (Elzaawely & Tawata, 2012), anticancer (Sadeghi-Aliabadi et al., 2008; Kaur & Kalia 2012b) stimulatory effect on the immune system (Bowait et al., 2010) and antibacterial (Ali et al., 2013).

W. calendulacea (Family Asteraceae) is a rare medicinal herb, used in Asian and South American countries that serves in traditional medicine. It has a long history of traditional use in revitalizing the liver and treating liver dysfunction. (Kirtikar & Basu, 1993; Murugaian et al., 2008). The phytochemical scans of the methanolic extract of *W. calandulaceae* showed a diverse set of bioactive compounds, including high concentrations of phenols, flavonoids, and significant quantities of saponins, phytosterols, alkaloids, terpenoids, and tannins. (Shahid et al., 2024). The ethanol extract of *W. calendulacea* is known for its anti-osteoporotic activity in the ovariectomized rat model of osteoporosis (Shirwaikar et al., 2010) due to the presence of isoflavones and wedelolactone, which are known to act as phytoestrogens and may be responsible for the antiosteoporotic activity (Shirwaikar et al., 2006). This plant also has neuroprotective, hepatoprotective, cytotoxicity, antibacterial and importantly reported to have anti-cancer potency. (Mottakin et al., 2004). Additionally, it is also used for the treatment of hepatic disorders and diarrhea and its leaves can be used in treatment of dermatological and digestive system disorders (Kanta et al., 2016).

Oxidative stress is defined by an imbalance between an increase in the level of prooxidants (free radicals) and antioxidants (Nemudzivhadi & Masoko, 2014). This imbalance can cause oxidative damage to the cellular structure and potentially destroy tissues and large biomolecules such as lipids, DNA, and proteins (Nemudzivhadi & Masoko,2014) contributing to the development of several human diseases, including neurodegenerative disorders such as Alzheimer's disease, obstructive pulmonary disease, atherosclerosis, diabetes, cardiovascular complications, certain types of cancers, and aging (Poulose et al., 2014; Singh et al., 2014). Phytochemical compounds of herbal medicinal plants have antioxidants, anti-inflammatory and anticancer activities support of their potential health benefits (Patra & Singh, 2018).

The antioxidative capacity (AC) is often used to characterize the health-promoting properties of various antioxidant phytochemicals products of vegetables fruits and medicinal plants. These compounds play an important role in the prevention and treatment of chronic diseases caused by oxidative stress (Soobrattee et al., 2005; Sung & Lee, 2010; Zhang et al., 2015). They often possess strong antioxidants and free radical scavenging abilities, as well as anticancer, anti-aging, and anti-inflammatory action, and protective action for diabetes mellitus cardiovascular diseases which are also the basis of other bioactivities and health benefits (Wu et al., 2012; Deng et al., 2012; Zhang et al., 2015). The determination of antioxidant potential is related to the action of a substance's capacity of protecting biological systems from adverse reactions which are caused by the excessive oxidation-induced reactive oxygen species (ROS). An increasing number of reports of the preventive role of antioxidants found in food have led to the development of a variety of assays measuring the antioxidant capacity (Prior et al., 2005; Krishnaiah et al., 2011). Some commonly used methods are based on peroxyl radical scavenging (ORAC), ferric tripyridyltriazine complex reduction (FRAP), organic radical scavenging (ABTS, DPPH), and Metal Chelation Activity. (Frankel and Meyer, 2000; Sanchez-Moreno, 2002; Sielicka et al. 2014).

We conducted this study to determine phytochemical screening using spectrophotometric assay, by HPLC and GC-MS analysis and to evaluate the antioxidant activity of methanolic leaves extracts of *M. leucadendra*, *C. arvensis* and *W. calenlulacea*.

2. MATERIALS AND METHODS

Preparation of filtrate of the used plants

All the chemicals and plants used were of analytical grade. The plants were purchased from the local market. The plant samples of *M. leucadendra*, *C. arvensis*, and *W. calenlulacea* leaves were immersed separately in 14 L of methanol for each plant and homogenized using UltraTurrax T50 IKA Labotechnik and shaft number G45ME for 15 minutes with a pause of 5 minutes each 3 minutes' interval at 6000 rpm. The resulting mixture was macerated for 24 hours and then filtered. The filtrate was collected and evaporated under a vacuum at 40°C.

Quantitative analysis of the different constituents of the three plants filtrates

2. A_1. Quantitative determination of *M. leucadendra*), *C. arvensis* and *W. calenlulacea* leaves extracts by spectrophotometric analysis.

i. Determination of total terpenoid compounds

The amount of 200 µl of plant extract or linalool was mixed with 2 ml of sodium carbonate and 1.5 ml of chloroform and then vortexed. A volume of 100 µl of sulfuric acid was added and the mixture was incubated for 2 hours at room temperature in the dark. The reddish-brown precipitate formed was decanted from the supernatant. Then, 1.5 ml of methanol (95%, v/v) was added to the precipitate, and the absorbance was measured at 538 nm (Ghorai et al, 2012).

ii. Determination of total steroids compounds

The volume of 200 µl of plant extract or cholesterol were mixed with 2 ml sodium carbonate and 0.80 ml of methanol, 0.35 ml vanillin and 1.25 ml sulfuric acid (72%). The tubes were incubated at 60℃ for 10 min, then cooled. The absorbance was measured at 544 nm (Moyo et al, 2013).

iii. Determination of total tannin compounds

Folin-Ciocalteau reagent (100 µl) and 2 ml sodium carbonate were added and mixed well with 100 µl of standard or sample (1 mg/1ml) or each concentration, the mixture was incubated at 25°C for 2hr. The absorbance of the resulting blue color solution was measured at 750 nm (Bizuayehu et al, 2016).

iv. Determination of total saponin content

A volume of 0.25 ml sample was added to 1 ml of reagent glacial acetic acid: sulfuric acid (1:1 v/v). The mixture was vortexed and incubated at 60 ℃ for 30 min then cooled. The absorbance of the sample was measured at 527 nm (Medina-Meza et al, 2016).

v. Determination of total alkaloid content

The plant methanolic extract sample was mixed with 1 ml of HCl and filtered off. One milliliter of the filtrate was transferred to a separating funnel and washed three times with 10 ml chloroform. The pH of this solution was adjusted to pH 7 using NaOH. Then five ml of bromocresol green (BCG) solution along with five ml of phosphate buffer were added to the neutralized solution of the extract or each standard concentration of berberine. Each mixture was shaken, and the formed complex was extracted with 1-, 2-, 3- and 4-ml chloroform by vigorous shaking. The organic layers were collected in 10 ml volumetric flask and diluted to volume with chloroform (10 ml). The absorbance of the yellow-colored complex in chloroform was measured at 470 nm (Shamsa, et al, 2008).

2. A_2. Quantitative determination of *M. leucadendra, C. arvensis,* and *W. calenlulacea* leaves extracts by HPLC.

HPLC analysis was used to identify and quantify the phenolic and flavonoid compounds present in *M. leucadendra*), *C. arvensis* and *W. calenlulacea* extract (Elbanoby et al, 2024). Each extract was accurately weighed and sonicated for 15 minutes, filtered using a 0.22 µm nylon syringe filter, and 10 µl was injected into the HPLC system.

2. A_3. GC-MS analysis to measure the totally different constituents of the three plants.

The chemical composition of samples was performed using a Trace GC-TSQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–5MS (30 m x 0.25 mm x 0.25 µm film thickness). The column oven temperature was initially held at 50°C and then increased by 5°C /min to 250°C with hold for 2 minutes. The temperature increased to the final temperature of 300°C by 30°C /min and held for 2 min. The injector and MS transfer line temperatures were kept at 270, and 260°C, respectively. Helium was used as a carrier gas at a constant flow rate of 1 ml/min. The solvent delay was 4 min and diluted samples of one µl were injected automatically using autosampler AS1300 coupled with GC in the split mode. EI mass spectra were collected at 70 eV ionization voltages over the range of m/z 50–650 in full scan mode. The ion source temperature was set at 200°C. The components were identified by comparison of their mass spectra with those of WILEY 09 and NIST 14 mass spectral databases (Abd El-Kareem et al., 2016).

B. Antioxidant activity studies of plant extracts

i. Oxygen radical absorbance capacity (ORAC)

The assay was carried out according to the method of Liang et al (2014). Briefly, ten µL of the prepared sample(s) was incubated with 30 µL fluoresceine (100 nM) for 10 min at 37°C. Fluorescence measurement (485 EX, 520 EM, nm) was carried out for three cycles (cycle time, 90 sec.). Afterward, 70 µL of freshly prepared 2,2'-Azobis(2-amidinopropane) dihydrochloride (AAPH) (300 mM) was added immediately to each well. Fluorescence measurement (485 EX, 520 EM, nm) continued for 60 min (40 cycles, every 90 Seconds).

ii. Ferric Reducing Antioxidant Power (FRAP)

The assay was carried out according to the method of Benzie & Strain (1996) with minor modifications to be carried out in microplates, A freshly prepared TPTZ reagent (300 mM Acetate Buffer (PH=3.6), 10 mM TPTZ in 40 HCl, and 20 mMFeCl3, in a ratio of 10:1:1 v/v/v, respectively). 190 µL from the freshly prepared TPTZ reagent were mixed with 10 µL of the sample in 96 wells plate (n=3), the reaction was incubated at room temp. For 30 min in the dark. At the end of the incubation period, the resulting blue color was measured at 593nm.

iii. 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging activity

The assay was carried out according to the method of Arnao et al, (2001) with minor modifications to be carried out in microplates briefly; 192 mg of ABTS were dissolved in distilled water and transferred to a 50 mL volumetric flask then the volume was completed with distilled water. 1mL of the previous solution was added to 17 µL of 140 mM potassium persulphate and the mixture was left in the dark for 24 hours. After that, 1mL of the reaction mixture was completed to 50 mL with methanol to obtain the final ABTS dilution used in the assay. 190 µL of the freshly prepared ABTS reagent were mixed with 10 µL of the sample/ compound in 96 wells plate (n=6), and the reaction was incubated at room temp. For 30min in the dark. At the end of incubation time, the decrease in ABTS color intensity was measured at 734 nm.

iv. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical assay was carried out according to the method of Boly et al. (2016). Briefly, 100µL of freshly prepared DPPH reagent (0.1% in methanol) was added to 100 µL of the sample in a 96-well plate (n=6), and the reaction was incubated at room temp for 30 min in dark. At the end of the incubation time, the resulting reduction in DPPH color intensity was measured at 540 nm.

v. Metal chelation activity

The assay was carried out according to the method of Santos et al. (2017) with minor modifications to be carried out in microplates, briefly; 20 µL of the freshly prepared ferrous sulfate (0.3 mM) was mixed with 50 µL of the sample/

compound in 96 wells plate (n=6). Afterward, 30 µL of ferrozine (0.8 mM) was added to each well. The reaction mixture was incubated at room temperature for 10 min. At the end of the incubation time, the decrease in the produced color intensity was measured at 562 nm.

3. RESULTS

3.A$_1$. Phytochemical composition of *M. leucadendra*, *C. arvensis,* and *W. calenlulacea* methanolic leaves extracts.

The phytochemical analysis of *M. leucadendra*, *C. arvensis,* and *W. calenlulacea* leaves extracts revealed the presence of bioactive compounds, including terpenoids, steroids, tannins, saponins, and alkaloids, which are likely to play a role in their therapeutic potentials, including antioxidants, anti-inflammatory, and anticancer effects **(Table 1)**.

The *M. leucadendra*, *C. arvensis*, and *W. calenlulacea* leaves extract contain some beneficial compounds, with steroids being the most abundant phytochemicals. These compounds are known for their anti-inflammatory and anticancer effects. Alkaloids and terpenoids are the second most abundant, playing a key role in their anti-inflammatory and anticancer properties. Terpenoids are also known for their strong antioxidant effects. The extracts also contain moderate amounts of saponins that play a key role in strong immune-modulating and anticancer properties. Even though tannins are present in lower concentrations, they still contribute to the extract's overall antioxidant and anticancer benefits.

Table 1. Phytoconstituents of *M. leucadendra, C. arvensis,* and *W. calenlulacea* leaves extract.

	Concentration mg/100 mg leaves extracts		
Phytochemical Component	*M. leucadendra*	*C. arvensis*	*W. calenlulacea*
Terpenoids (as mg Linalool)	1.350	1.543	1.109
Steroids (as mg Cholesterol)	16.731	20.205	19.543
Tannins (as µg Gallic acid)	0.0268	0.0241	0.000625
Saponins (as mg Sapogenin)	0.226	0.374	0.374
Alkaloids (as mg Berberine)	5.808	4.309	3.959

3.A$_2$. HPLC analysis of *M. leucadendra, C. arvensis,* and *W. calenlulacea* leaves extracts.

HPLC analysis was used to identify and quantify the phenolic and flavonoid compounds present in *M. leucadendra*, *C. arvensis,* and *W. calenlulacea* extracts. Gallic acid and rutin were detected in *M. leucadendra* and *C. arvensis* extracts. Gallic acid, quercetin, and kaempferol were detected in *M. leucadendra* extract, while chlorogenic acid, apigenin, and caffeic acid were detected in *C. arvensis* extract. On the other hand, all the mentioned phenolic and flavonoid compounds are not detected in *W. calenlulacea* (**Table 2a**). HPLC analysis of leaves extract showed a high content of rutin, gallic acid, quercetin, and kaempferol in *M. leucadendra* extract (**Table 2b** & **Figure 1**), while other compounds such as catechin, chlorogenic acid, ellagic acid, hesperidin, apigenin, and caffeic acid were not detected in *M. leucadendra* extract. HPLC analysis of *C. arvensis* leaves extract showed a high content of caffeic acid, apigenin, and rutin (**Table 2c** & **Figure 2**). The major compounds detected in the *M. leucadendra* extract were rutin and gallic acid, which accounted for more than 90% of the total area (**Table 2b & Figure 1**). While rutin and caffeic acid were the major compounds detected in the *C. arvensis* leaves extract which accounted for more than 85 % of the total area (**Table 2c & Figure 2**). These results indicate the rich presence of gallic acid as a bioactive phenolic in the *M. leucadendra* extract.

Table 2a. Phenolics and flavonoids composition of *M. leucadendra, C. arvensis,* and *W. calenlulacea* leaves extract based on HPLC analysis.

Reference compounds	Gallic acid	Catechin	Chlorogenic acid	Rutin	Ellagic acid	Hesperidin	Quercetin	Kampeferol	Apigenin	Caffeic acid
Melaleuca leucadendra	Detected	Not Detected	Not Detected	Detected	Not Detected	Not Detected	Detected	Detected	Not Detected	Not Detected
Convolvulus arvensis	Not Detected	Not Detected	Detected	Detected	Not Detected	Not Detected	Not Detected	Not Detected	Detected	Detected
Waldelia calenlulacea	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected

Table 2b: Phenolics and flavonoids composition of *Melaleuca leucadendra* (ML) leaves extract based on HPLC analysis.

Compound	Concentration (mg/g)	Retention Time (RT)	% Area
Gallic acid	1.51 ± 0.0007	13.375	75.17
Rutin	1.77 ± 0.003	48.455	17.50
Quercetin	0.30 ± 0.0008	57.491	6.05
Kaempferol	0.45 ± 0.0019	59.636	1.28

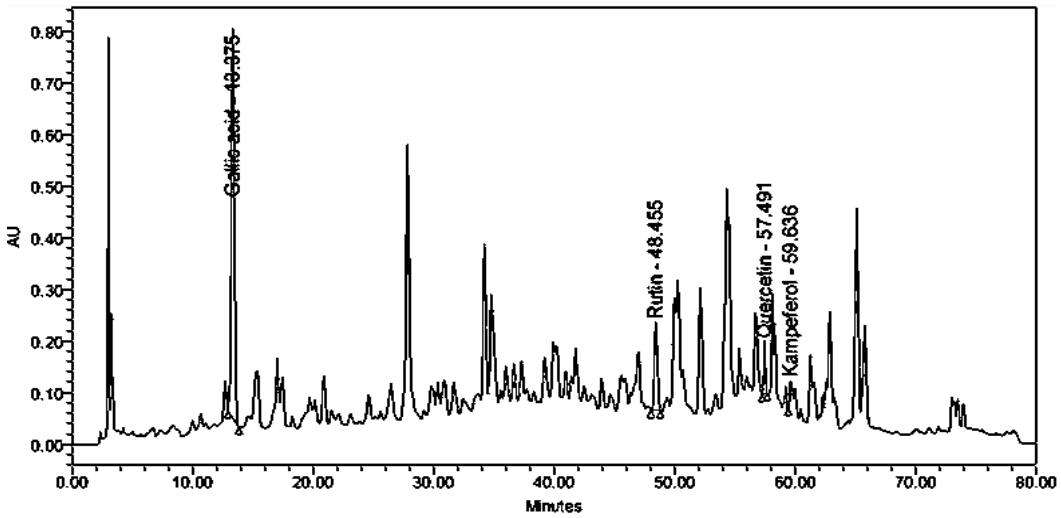

Figure 1. HPLC analysis of *M. leucadendra* leaves extract.

Table 2c. Phenolics and flavonoids composition of *C. arvensis* leaves extract based on HPLC analysis.

Compound	Concentration (mg/g)	Retention Time (RT)	% Area
Chlorogenic acid	0.074 ± 0.0001	31.422	1.15
Caffeic acid	0.131 ± 0.002	33.703	6.46
Rutin	1.868 ± 0.0004	48.472	79.11
Apigenin	0.587 ± 0.0001	60.112	3.28

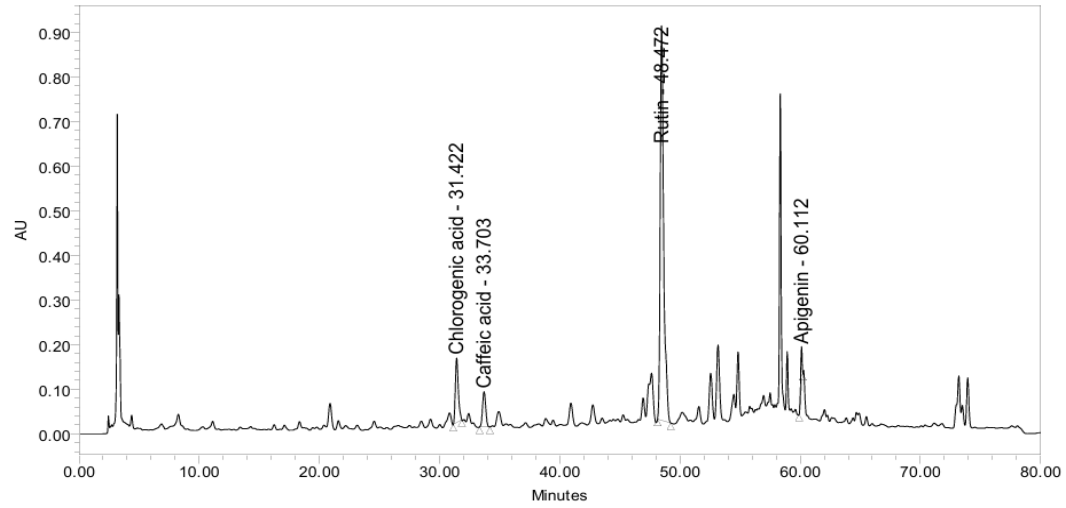

Figure 2. HPLC analysis of *C. arvensis* leaves extract.

3.A$_3$. GC-MS analysis of plant extracts

A. GC-MS analysis profiling of *M. Leucadendra* leaves extract.

More than 39 compounds belonging to different chemical families were identified from the GC-MS analysis of the methanolic extract of *M. Leucadendra* that exhibits various phytochemical activities. The chromatogram is presented in **Figure 3**, while the chemical constituents with their retention time (RT), molecular formula, molecular weight (MW), and concentration (%) are presented in Table 3. The retention times range from 12.45 to 38.67 minutes. The most prominent peaks were observed at retention times of 23.24, 24.91, 26.43, 27.72, and 38.67 minutes, with the highest concentration % corresponding to the retention time of 38.67 minutes. 9,19-Cyclolanostan-3-ol, 24-methylene-, (3á)- is most abundant (18.08%) in extract and has a more complex steroidal structure while 4H-1-Benzopyran-4-one, 2-(3,4-dimethoxyphenyl)-3,5-dihydroxy-7-methoxy- compound has the lowest concentration at 0.31%.

The extract of *M. leucadendra* contains a variety of bioactive chemicals with potential therapeutic qualities such as antibacterial, anti-inflammatory, and potentially anticancer effects, which may be exerted by the presence of fatty acid esters, aziridine derivatives, and other bioactive components.

Table 3. . Phytochemical profiling of M. leucadendra leaves extract using GC-MS spectral analysis

No	RT	Conc. %	Molecular Formula	Molecular Weight	Compound Name	Family
1	12.45	0.39	$C_{12}H_{15}N$	173	Aziridine, 1-(1,2,3,4-tetrahydro-2-naphthyl)-	Aziridines
2	12.90	0.57	$C_{14}H_20$	188	2,2,9,9-Tetramethyldec-5-ene-3,7-diyne	Alkynes
3	14.70	0.99	$C_{13}H_{26}O_2$	214	Undecanoic acid, 10-methyl-, methyl ester	Fatty Acid Esters
4	14.96	0.68	$C_{17}H_{32}O_2$	268	10-Methyl-8-tetradecen-1-ol acetate	Acetates
5	15.45	0.50	$C_{17}H_{32}O_2$	268	7-Hexadecenoic acid, methyl ester, (Z)-	Fatty Acid Esters
6	16.46	1.36	$C_{10}H_{11}NS$	177	4H-Thieno[3,2-b] indole, 5,6,7,8-tetrahydro-	Thienoindoles
7	19.11	1.87	$C_{15}H_{30}O_2$	242	Methyl tetradecanoate	Fatty Acid Esters
8	20.62	1.45	$C_{14}H_{28}O_2$	228	Tetradecanoic acid	Fatty Acids
9	21.32	0.88	$C_{20}H_{40}O_2$	312	Ethanol, 2-(9-octadecenyloxy)-, (Z)-	Alcohols
10	21.53	1.03	$C_{17}H_{32}O_2$	268	7-Methyl-Z-tetradecen-1-ol acetate	Acetates
11	21.82	0.37	$C_{15}H_{20}O_5$	280	Tetraneurin - A - Diol	Diols
12	22.09	1.15	$C_{23}H_{36}O_4$	376	Phthalic acid, butyl undecyl ester	Phthalates
13	22.16	0.62	$C_{16}H_{12}O_6$	300	4H-1-benzopyran-4-one, 5,7-dihydroxy-2-(4-hydroxyphenyl)-3-methoxy	Glycolipid Derivatives
14	22.69	0.54	$C_{18}H_{30}D_6O$	274	2,2,3,3,4,4 Hexadeutero octadecanal	Deuterated Compound
15	23.24	13.30	$C_{17}H_{34}O_2$	270	Hexadecanoic acid, methyl ester	Fatty Acid Esters
16	24.91	10.31	$C_{16}H_{32}O_2$	256	Hexadecanoic acid	Fatty Acids
17	26.26	2.14	$C_{19}H_{34}O_2$	294	9,12-Octadecadienoic acid, methyl ester, (E, E)-	Fatty Acid Esters
18	26.43	9.20	$C_{19}H_{36}O_2$	296	9-Octadecenoic acid (Z)-, methyl ester	Fatty Acid Esters
19	26.53	2.55	$C_{19}H_{36}O_2$	296	10-Octadecenoic acid, methyl ester	Fatty Acid Esters
20	26.89	5.61	$C_{19}H_{38}O_2$	298	Octadecanoic acid, methyl ester	Fatty Acid Esters
21	26.99	0.50	$C_{19}H_{34}D_4O_2$	302	Methyl-9,9,10,10-D4-octadecanoate	Fatty Acid Esters
22	27.18	0.80	$C_{19}H_{34}O_6$	358	Dodecanoic acid, 2,3-bis(acetyloxy)propyl ester	Fatty Acid Esters
23	27.72	9.02	$C_{18}H_{34}O_2$	282	Trans-13-Octadecenoic acid	Fatty Acids
24	28.12	4.48	$C_{18}H_{36}O_2$	284	Octadecanoic acid	Fatty Acids
25	28.54	0.56	$C_{28}H_{44}O_4$	444	9-Octadecenoic Acid, (2-Phenyl-1,3-Dioxolan-4-Yl) Methyl Ester, Cis-	Fatty Acid Esters
26	29.08	0.48	$C_{30}H_{52}O_3Si$	488	9,10-Secocholesta-5,7,10(19)-Triene-1,3-Diol, 25-[(Trimethylsilyl)Oxy]-,(3á,5Z,7E)-	Steroids
27	29.77	0.43	$C_{18}H_{34}O_2$	282	9-Octadecenoic Acid (Z)-	Fatty Acids
28	29.96	0.69	$C_{17}H_{32}O_2$	268	7-Methyl-Z-Tetradecen-1-Ol Acetate	Acetates
29	30.24	0.79	$C_{25}H_{42}O_2$	374	Cyclopropanebutanoic acid, 2-[[2−[[2−[(2−pentyl cyclopropyl)methyl]cyclopropyl]methyl]cyclopropyl]methyl]−,methylester[[2−[[2−[(2−pentyl cyclopropyl)methyl]cyclopropyl]methyl]cyclopropyl] methyl]−, methyl ester	Cyclopropane Derivatives
30	30.67	0.55	$C_{19}H_{38}O_4$	330	Hexadecanoic acid, 2,3-dihydroxypropyl ester	Fatty Acid Esters
31	31.15	0.76	$C_{30}H_{52}O_3Si$	488	1,25-Dihydroxy vitamin D3, TMS derivative	Steroids
32	32.79	0.57	$C_{19}H_{22}O_6$	346	Isochiapin B	Flavonoids
33	33.36	0.85	$C_{23}H_{46}O_2$	354	Docosanoic acid, methyl ester	Fatty Acid Esters
34	33.67	2.43	$C_{24}H_{38}O_4$	390	1,2-Benzenedicarboxylic acid	Aromatic Acids
35	35.79	1.96	$C_{35}H_{70}$	490	17-Pentatriacontene	Alkenes
36	36.26	0.31	$C_{18}H_{16}O_7$	344	4H-1-Benzopyran-4-one, 2-(3,4-dimethoxy phenyl)-3,5-dihydroxy-7-methoxy-	Flavonoids
37	37.83	0.59	$C_{26}H_{44}O_5$	436	Ethyl iso-allocholate	Bile Acid Derivatives
38	38.13	0.65	$C_{37}H_{76}O$	536	1-Heptatriacotanol	Alcohols
39	38.67	18.08	$C_{31}H_{52}O$	440	9,19-Cyclolanostan-3-ol, 24-methylene-, (3á)-	Steroids

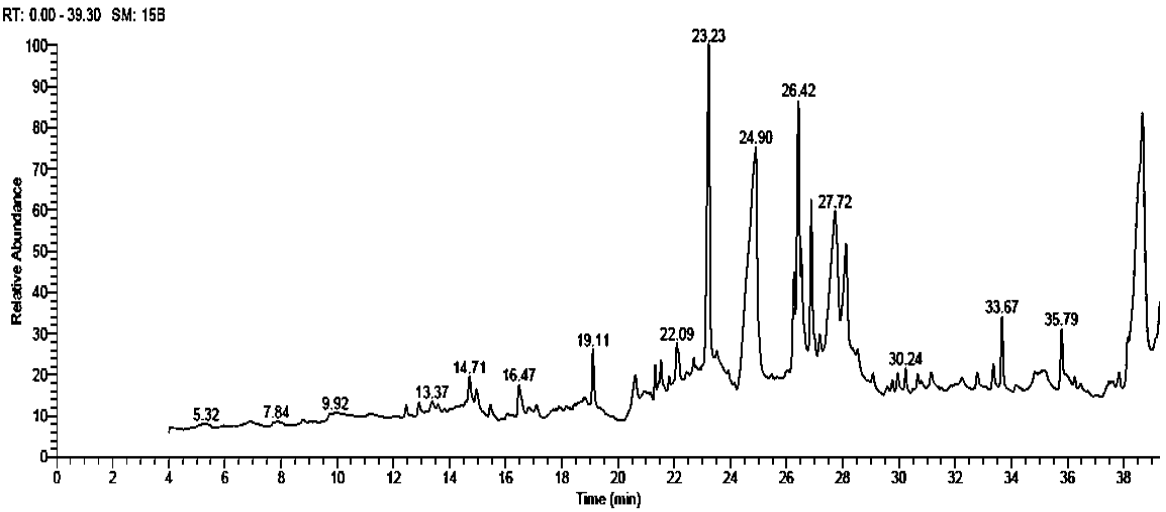

Figure 3. GC–MS chromatogram of *M. leucadendra* leaves extract.

B. GC-MS analysis profiling of *C. arvensis* extract

Many compounds belonging to different chemical families were identified from the GC-MS analysis of methanolic extract of *C. arvensis*. The chromatogram is presented in **Figure 4**, while the chemical constituents with their retention time (RT), molecular formula, molecular weight (MW), and concentration (%) are presented in **Table 4**. The retention times (RT) range from 10.35 to 38.75 minutes. The most prominent peaks were observed at retention times of 23.40, 25.66, 28.92, and 37.11, minutes, with the highest concentration % corresponding to the retention time of 37.11 minutes. Bicyclo[4.1.0]heptan-2-ol,1á-(3-methyl-1,3-butadienyl)-2à,6á dimethyl-3á-acetoxy is the most abundant (13.38%) in extract and has bicyclic monoterpene derivatives structure while 9-Octadecenoic acid compound has the lowest concentration at 0.20%.

The extract of *C. arvensis* contains a variety of bioactive chemicals with potential therapeutic qualities, including antibacterial, and anti-inflammatory effects, as evidenced by the presence of fatty acid esters, monoterpenoids, steroid derivatives, and other bioactive components.

Table 4. Phytochemical profiling of C. arvensis leaves extract using GC-MS spectral analysis

No	RT	Conc. %	Molecular formula	Molecular weight	Compound	Family Class Family Class
1	10.35	0.50	$C_{10}H_{16}O_2$	168	1-Oxaspiro[2.5]octan-4-one, 2,2,6-trimethyl-, cis-	Menthane Monoterpenoids
2	13.34	0.55	$C_{15}H_{26}O$	222	à-acorenol	Tertiary Alcohols
3	13.91	0.76	$C_{15}H_{24}$	204	1,1,4,7-Tetramethyl-1A,2,3,5,6,7,7A, 7B-octahydro-1H-cyclopropa [E]azulene	Sesquiterpenes
4	14.74	0.44	$C_{13}H_{26}O_2$	214	Dodecanoic acid, methyl ester	Fatty Acid Methyl Esters
5	15.94	0.28	$C_{21}H_{34}O_2$	318	5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)-	Fatty Acid Methyl Esters
6	16.58	0.32	$C_{18}H_{30}O_2$	278	10-Heptadecen-8-ynoic acid, methyl ester, (E) -	Fatty Acid Methyl Esters
7	16.69	0.35	$C_{19}H_{38}O_4$	330	Hexadecenoic acid, 2,3-dihydroxypropyl ester	Fatty Acid propyl Esters
8	17.55	0.30	$C_{24}H_{36}O_2$	356	9,10-Secochola-5,7,10(19)-trien-24-al, 3-hydroxy-, (3á,5Z,7E)-	Steroids
9	17.67	0.34	$C_{28}H_{48}O$	400	Cholestan-3-ol, 2-methylene-, (3á,5à)-	Steroids
10	19.15	1.14	$C_{15}H_{30}O_2$	268	Tetradecanoic acid, methyl ester	Fatty Acid Methyl Esters
11	19.55	0.40	$C_{16}H_{28}O_2$	252	1H-1-Inden-1,2,4,5,6,7,7a-hexahydro-7-(1-methylethoxy)	indenols
12	19.97	0.41	$C_{15}H_{22}O_{24}$	234	4-(3,3-Dimethyl-but-1-ynyl)-4-hydroxy-2,6,6-trimethylcyclohex-2-enone	Sesquiterpenoids
13	20.98	1.59	$C_{14}H_{28}O_2$	228	Tetradecanoic acid	Fatty Acid

14	21.11	0.28	$C_{15}H_{24}O_3$	252	Spiro[tricyclo[4.4.0.0(5,9)]decane-10,2'-oxirane],1-methyl-4-isopropyl-7,8-dihydroxy-, (8S)-	Sesquiterpenoids
15	21.39	1.11	$C_{20}H_{40}O_2$	312	Ethanol, 2-(9-octadecenyloxy)-, (Z)-	Ethoxylates of aliphatic alcohols
16	21.60	0.67	$C_{18}H_{36}O$	268	2-Pentadecanone, 6,10,14-trimethyl-	Sesquiterpenoids
17	21.87	0.28	$C_{17}H_{32}O$	252	13-Heptadecyn-1-ol	Long-Chain Fatty Alcohols
18	22.21	0.87	$C_{20}H_{40}O_2$	312	Ethanol, 2-(9-octadecenyloxy)-, (Z)-	ether
19	23.30	7.89	$C_{17}H_{34}O_2$	270	Hexadecanoic acid, methyl ester	Fatty Acid Methyl Esters
20	24.59	0.32	$C_{19}H_{38}O_4$	330	Hexadecanoic acid,2,3-dihydroxypropyl ester	Fatty Acid Propyl Esters
21	25.66	10.33	$C_{16}H_{32}O_2$	256	n-Hexadecanoic acid	Fatty Acid
22	26.38	1.86	$C_{19}H_{34}O_2$	294	9,12-Octadecadienoic acid, methyl ester, (E, E)-	Fatty Acid Methyl Esters
23	26.57	5.15	$C_{19}H_{36}O_2$	296	9-Octadecenoic acid (Z)-, methyl ester	Fatty Acid Methyl Esters
24	26.66	0.92	$C_{19}H_{36}O_2$	296	10-Octadecenoic acid, methyl ester	Fatty Acid Methyl Esters
25	26.78	0.20	$C_{18}H_{34}O_2$	282	9-Octadecenoic acid	Fatty Acid
26	27.00	2.83	$C_{19}H_{38}O_2$	298	Octadecanoic acid methyl ester	Fatty Acid Methyl Esters
27	27.29	0.38	$C_{27}H_{44}O_3$	416	9,10-Secocholesta-5,7,10(19)-triene 3,25,26-triol, (3á,5Z,7E) -	Steroid
28	27.88	1.05	$C_{20}H_{36}O_2$	308	Linoleic acid ethyl ester	Fatty Acid Ethyl Esters
29	28.43	0.36	$C_{18}H_{34}O_2$	282	cis-Vaccenic acid	Fatty Acid
30	28.92	9.51	$C_{18}H_{34}O_2$	282	cis-13-Octadecenoic acid	Fatty Acid
31	29.12	4.54	$C_{18}H_{36}O_2$	284	Octadecanoic acid	Fatty Acid
32	29.27	0.60	$C_{18}H_{34}O_3$	298	Oxiraneoctanoic acid, 3-octyl-, cis-	Fatty Acid
33	29.65	3.23	$C_{20}H_{34}O_8$	402	Tributyl acetyl citrate	Acetyl tributyl citrate
34	29.81	0.17	$C_{18}H_{34}O_2$	282	9-Octadecenoic acid (Z)-	Fatty Acid
35	30.21	5.68	$C_{17}H_{30}O_2$	266	4a,7,7,10a-Tetramethyldo decahydrobenzo[f]chromen-3-ol	triterpenoids
36	30.44	0.48	$C_{19}H_{36}O_3$	312	Oxiraneundecanoic acid, 3-pentyl-, methyl ester, trans	Fatty Acid Methyl Esters
37	30.92	0.68	$C_{23}H_{34}O_5$	390	3,14,16-Trihydroxycard-20(22)-enolide	Steroid
38	31.07	0.86	$C_{29}H_{50}O$	414	Stigmast-5-en-3-olL, (3á,24S)-	Steroid
39	31.52	1.29	$C_{20}H_{40}O_2$	312	Eicosanoic acid	Fatty Acid
40	32.93	0.57	$C_{19}H_{22}O_6$	346	Isochiapin B	Sesquiterpenes
41	33.14	0.46	$C_{21}H_{40}O_3$	340	Octadecanoic acid, 9,10-epoxy-, isopropyl ester	Fatty Acid Propyl Esters
42	33.51	0.72	$C_{23}H_{46}O_2$	354	Docosanoic acid, methyl ester	Fatty Acid Methyl Esters
43	33.84	1.49	$C_{24}H_{38}O_4$	390	Diisooctyl phthalate	Benzoic Acid Esters.
44	34.07	1.67	$C_{19}H_{30}O_2$	290	10a,12a-Dimethyl-hexadecahydro-2-oxa-chrysen-3-one	Steroids
45	34.42	1.07	$C_{18}H_{16}O_7$	344	4H-1-Benzopyran-4-one, 2-(3,4-dimethoxyphenyl)-3,5-dihydroxy-7-methoxy-	Flavonoids
46	35.53	2.17	$C_{29}H_{50}O_2$	430	2,5,7,8-Tetramethyl-2-(4,8,12trimethyltriecyl)-6-chromanol	Tocopherol
47	35.84	0.59	$C_{32}H_{66}$	450	Dortiacontane	Hydrocarbon
48	36.40	0.79	$C_{18}H_{36}O_4$	316	Erythro-9,10-dihydroxyoctadecanoic acid	Fatty Acid
49	36.72	4.80	$C_{24}H_{38}O_4$	390	1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	Phthalates
50	37.11	13.38	$C_{16}H_{24}O_3$	264	Bicyclo[4.1.0]heptan-2-ol,1á-(3-methyl-1,3-butadienyl)-2à,6ádimethyl-3á-acetoxy	Bicyclic Monoterpene Derivatives

51	37.66	1.69	$C_{20}H_{26}O_5$	346	2-Butenoic acid, 2-methyl-,dodecahydro-8-hydroxy-8a-methyl-3,5-bis(methylene)-2-oxonaphtho[2,3b]furan-4-yl ester	sesquiterpenoids
52	38.39	0.31	$C_{29}H_{50}O$	414	Stigmast-5-en-3-ol, (3á,24S)-	Steroids
53	38.75	0.64	$C_{32}H_{54}O_4$	502	7,8-Epoxylanostan-11-ol, 3-acetoxy-	Steroids

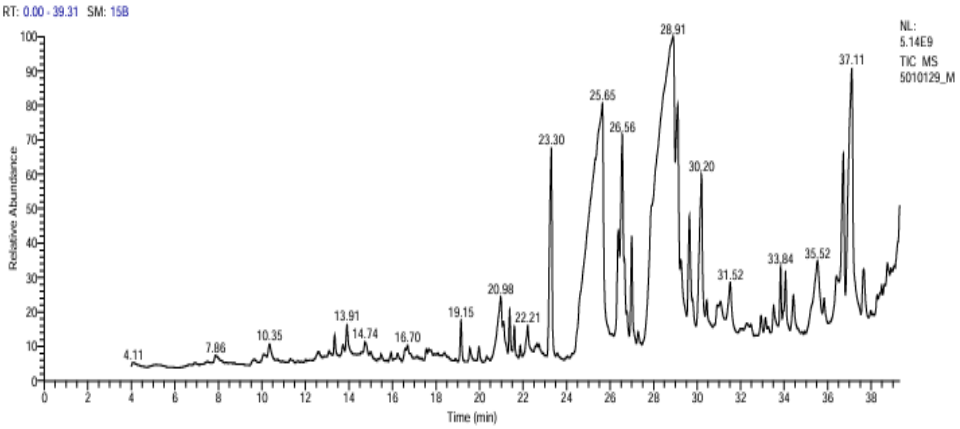

Figure 4. GC–MS chromatogram of *C. arvensis* leaves extract.

C. GC-MS analysis profiling of *W. calenlulacea* extract

More than 70 compounds belonging to different chemical families were identified from the GC-MS analysis of the methanolic extract of *W. calenlulacea*. The chromatogram is presented in **Figure 5**, while the chemical constituents with their retention time (RT), molecular formula, molecular weight (MW), and concentration (%) are presented in **Table 5**. The retention times (RT) range from 4.03 to 38.84 minutes. The most prominent peaks were observed at retention times of 25.20, 26.63, 26.50, 27.92, and 37.60 minutes, with the highest concentration % corresponding to the retention time of 26.63 minutes. 7H-furo[3,2-G][1]benzopyran-7-one, 4-methoxy- is most abundant (10.87%) in extract and has a more complex steroidal structure while 16-Nitrobicyclo[10.4.0]hexadecan-1-ol-13-one compound has the lowest concentration at 0.16%.

The extract of *W. calenlulacea* appears to include a variety of bioactive chemicals with potential therapeutic qualities, including antibacterial, and anti-inflammatory effects, as evidenced by the presence of fatty acid esters, Sesquiterpenoids, steroids, and other bioactive components.

Table 5. Phytochemical profiling of W. calenlulacea leaves extract using GC-MS spectral analysis

No	RT	Conc. %	Molecular Formula	Molecular Weight	Compound	Family Class
1	4.03	0.36	$C_{18}H_{32}O_2$	280	17-Octadecynoic acid	Fatty Acid
2	10.00	1.06	$C_{10}H_{16}O_2$	168	7-Oxabicyclo[4.1.0]heptan-2-one, 6-methyl-3-(1-methylethyl)-	oxygenated monoterpenes
3	10.56	0.31	$C_{11}H_{10}O_2$	174	4-Hydroxy-4-methyl-4H-naphthalen-1-one	Terpenoid
4	11.56	0.25	$C_{13}H_{18}O$	190	1-Naphthalenol, 1,2,3,4-tetrahydro-2,5,8-Trimethyl-	Sesquiterpenoids
5	12.02	0.24	$C_12H_{10}O_4S$	250	4,4'-Dihyroxydiphenylsulphone	bisphenol S
6	13.05	0.16	$C_{16}H_{27}NO_4$	297	16-Nitrobicyclo[10.4.0]hexadecan-1-ol-13-one	cyclohexenones
7	13.60	0.22	$C_{19}H_{30}O_2$	290	Methyl 8,10-octadecadiynoate	Fatty Acid Methyl Ester
8	13.85	0.32	$C_{16}H_{26}O_2$	250	(1,2,3,4,5,6,7,8-octahydro-3,8,8-trime thylnaphth-2-yl)methyl ester	Sesquiterpenoids
9	14.71	0.42	$C_{13}H_{26}O_2$	214	Dodecanoic acid, methyl ester	Fatty Acid Methyl Ester
10	15.01	0.51	$C_{14}H_{24}O_3$	240	Octahydrobenzo[b]pyran, 4a-acetoxy-5,5,8a-trimethyl-	Sesquiterpenoid

11	15.47	0.18	$C_{14}H_{22}O_2$	222	(4-Methoxymethoxy-hex-5-ynyliden e)-cyclohexane	Alkyne Derivative
12	15.92	0.17	$C_{12}H_{20}O_2$	196	(7R,8R)-cis-anti-cis-Tricyclo[7.3.0.0(2,6)] dodecane-7,8-diol	Dicyclic Diol
13	16.08	0.26	$C_{15}H_{24}O_2$	236	Limonen-6-ol, pivalate	Terpenoid Ester
14	16.23	0.22	$C_{14}H_{20}O_3$	236	5,6,6-Trimethyl-5-(3-oxobut-1-enyl)-1-oxaspiro [2.5]octan-4-one	Oxygenated Terpenoid
15	16.50	0.70	$C_9H_9F_3O_2$	206	Phen-1,4-diol, 2,3-dimethyl-5-Trifluoromethyl-	Phenolic Derivative
16	16.95	0.39	$C14H_{22}O_2$	222	(4-Methoxymethoxy-hex-5-ynyliden e)-cyclohexane	Terpenoid
17	17.17	0.30	$C_{14}H_{24}O$	208	2-Methyl-4-(2,6,6-trimethylcyclohex-1-enyl)but-2-en-1-ol	Terpenoids
18	17.76	0.33	$C_{20}H_{30}O_2$	302	cis-5,8,11,14,17-Eicosapentaenoic acid	Fatty Acid
19	17.90	0.22	$C_{10}H_{12}N_2O$	176	1H-indol-5-ol, 3-(2-aminoethyl)	Indole Alkaloid
20	18.89	0.38	$C_{14}H_{20}O_3$	236	5,6,6-Trimethyl-5-(3-oxobut-1-enyl) 1-oxaspiro[2.5]octan-4-one	Fatty Acid Methyl Ester
21	19.12	1.28	$C_{15}H_{30}O_2$	242	Methyl tetradecanoate	Fatty Acid
22	20.10	0.99	$C_{12}H_{12}N_2O_3$	232	1, 4-Dihydro-1-ethyl-7methyl-4-oxo-1,8-naphthyridine-3-carboxylic acid	Fatty Acid
23	20.85	0.90	$C_{14}H_{28}O_2$	228	Tetradecanoic acid	Fatty Acid
24	21.20	0.17	$C_{15}H_{30}O_2$	242	Pentadecanoic acid	Fatty Acid
25	21.40	4.25	$C_{20}H_{38}$	278	2,6,10-Trimethyl,14-ethylene-14-pentadecne	pentadecane
26	21.62	1.19	$C_{18}H_{34}O_3$	298	Oxiraneoctanoic acid, 3-octyl-, cis-	Epoxy Fatty Acid
27	21.91	1.05	$C_{18}H_{36}O$	268	2-Pentadecanone, 6,10,14-trimethyl-	Ketone
28	22.10	0.70	$C_9H_{14}O_2S$	168	2-Thia-adamantane-4,8-diol	Sulfur-containing Terpenoid
29	22.30	2.29	$C_{11}H_6O_3$	186	2H-Furo[3,2-H][1]benzopyran-2-one	Coumarin
30	22.47	4.95	$C_{11}H_6O_3$	186	7H-Furo[3,2-G][1]benzopyran-7-one	Coumarin
31	23.08	0.45	$C_{16}H_{26}O_2$	250	Formic acid, 3,7,11-trimethyl-1,6,10-dodecatrien-3-yl ester	Esterified Terpenoid
32	23.30	6.43	$C_{17}H_{34}O_2$	270	Hexadecanoic acid, methyl ester	Fatty Acid Methyl Ester
33	23.59	0.31	$C_5H_8C_lN_5$	173	1,3,5-Triazine-2,4-diamine, 6-chloro-N-ethyl	Fatty Acid Methyl Ester
34	23.99	0.12	$C_{19}H_{34}O_6$	358	Dodecanoic acid, 2-(Acetyloxy)-1-[(acetyloxy)methyl] ethyl ester	Fatty Acid Methyl Ester
35	24.60	0.20	$C_{19}H_{38}O_4$	330	Hexadecenoic acid, 2,3-Dihydroxypropyl ester	Fatty Acid Methyl Ester
36	25.20	5.92	$C_{16}H_{32}O_2$	256	n-Hexadecanoic acid	Fatty Acid
37	26.05	0.30	$C_{16}H_{28}O_3$	268	Z-(13,14-epoxy)tetradec-11-en-1-ol acetate	Epoxy Fatty Alcohol Ester
38	26.29	0.44	$C_{19}H_{34}O_2$	294	7,10-Octadecadienoic acid, methyl ester	Fatty Acid Methyl Ester
39	26.50	6.39	$C_{12}H_8O_4$	216	5-Methoxy-2H-furo[2,3-H]chromen-2-one	Methoxycoumarin
40	26.63	10.87	$C_{12}H_8O_4$	216	7H-furo[3,2-G][1]benzopyran-7-one, 4-methoxy-	Methoxycoumarin
41	26.96	2.21	$C_{19}H_{38}O_2$	298	Octadecanoic acid, methyl ester	Fatty Acid Methyl Ester
42	27.26	0.39	$C_{15}H_{22}O_2$	234	-2a,4,5,5a,6,7,8,9b-octahydro-2H-nap htho[1,2-b]oxireno[2,3-c]furan	hHeterocyclic containing oxirane, furan
43	27.92	5.44	$C_{18}H_{34}O_2$	282	9-Octadecanoic acid (Z)-	Oleic acid
44	28.04	4.36	$C_{18}H_{30}O_2$	278	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	Unsaturated fatty acid
45	28.38	3.94	$C_{18}H_{36}O_2$	284	Octadecanoic acid	Fatty Acid

46	28.62	0.14	$C_{28}H_{46}O$	٣٩٨	Cholesta-8,24-dien-3-ol, 4-methyl-, (3á,4à)	Steroid
47	29.15	0.29	$C_{37}H_{76}O$	536	1-Heptatriacotanol	Long-chain Alcohol
48	29.45	0.18	$C_{26}H_{44}O_5$	٤٣٦	Ethyl iso-allocholate	ethyl iso-allocholate
50	29.67	0.25	$C_{32}H_{64}O_3$	496	Palmitic acid, 2-(tetradecyloxy) ethyl ester	Fatty Acid Ethyl Ester
51	29.74	0.10	$C_{30}H_{52}O_3Si$	488	9,10-Secocholesta-5,7,10(19)-triene-1,3-diol, 25-[(trimethylsilyl)oxy]-, (3á,5Z,7E)	1,25-Dihydroxyvitamin D3
52	29.81	0.16	$C_{18}H_{16}O_7$	344	4H-1-Benzopyran-4-one, 2-(3,4-dimethoxy-phenyl)- 3,5 dihydroxy-7-methoxy-	chroman-4-one fused 1,3,4-thiadiazole derivatives
53	30.02	0.48	$C_{23}H_{30}N_2O_5$	414	Aspidospermidin-17-ol, 1-acetyl-19,21-epoxy-15,16-dimethoxy	Alkaloid
54	30.27	0.90	$C_{19}H_{36}O_3$	312	Oxiraaneundecanic acid, 3-pentyl-, methyl ester, trans	Fatty Acid Methyl Ester
55	30.64	0.20	$C_{20}H_{28}O_6$	364	1H-2,8a-Methanocyclopenta[a]cyclopropa[e]cyclodecen-11-one, 1a,2,5,5a,6,9,10,10a-octahydro-5,5a,6-trihydroxy-1,4-bis(hydroxymethyl)-1,7,9-trimethyl-	Polycyclic hydrocarbon derivative
56	30.76	1.52	$C_{21}H_{40}O_2$	324	4,8,12,16-Tetramethylheptadecan-4 olide	Hydrocarbon
57	31.15	1.07	$C_{21}H_{36}O_4$	352	Linolenic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (Z,Z,Z)	Fatty Acid propyl Ester
58	31.28	1.18	$C_{19}H_{38}O_4$	330	Hexadecanoic acid, 2,3-Dihydrxypropyl ester	Fatty Acid Methyl Ester
59	33.40	0.83	$C_{23}H_{46}O_2$	354	Docosanoic acid, methyl ester	Fatty Acid Methyl Ester
61	33.72	1.67	$C_{24}H_{38}O_4$	390	1,2-Benzenedicarboxylic acid	1,2-Benzenedicarboxylic acid
62	33.96	0.33	$C_{30}H_{52}O_3Si$	488	9,10-Secocholesta-5,7,10(19)-triene-1,3-diol, 25-[(trimethylsilyl)oxy]-, (3á,5Z,7E)-	Steroids
63	34.24	0.63	$C_{30}H_{52}O_3Si$	488	1,25-Dihydroxyvitamin D3, tms derivative	Steroids
64	34.85	0.22	$C_{22}H_{42}S_2Si$	398	t-Butyl-(2-[3-(2,2-dimethyl-6-methylene-cyclohexyl)-propyl]-[1,3]dithian-2-yl)-dimethyl-silane	Dimethylsilane derivative
65	35.76	0.58	$C3_2H_{66}$	450	Dotriacontane	Hydrocarbons
66	36.14	0.43	$C_{26}H_{44}O_5$	436	Ethyl iso-allocholate	ethyl iso-allocholate complex
67	36.29	0.57	$C_{24}H_{46}O_2$	366	Cyclopropanedodecanoic acid, 2-octyl-, methyl ester	Fatty Acid Methyl Ester
68	36.52	0.22	$C_{34}H_{68}O_5S$	584	3-(Tetradecanoyloxy)-2-[(trimethylsilyl)oxy]-propyl myristate	Fatty esters
69	37.11	0.39	$C_{26}H_{44}O_5$	436	Ethyl iso-allocholate	ethyl iso-allocholate complex
70	37.60	6.26	$C_{30}H_{50}$	410	2,6,10,14,18,22-Tetracosahexane, 2,6,10,15,19,23-hexamethyl-	Squalene
71	37.93	3.41	$C_{27}H_{34}NO_4$	436	(+)-(P,1R,3S)-5-(4,5-Dimethoxy-2-methyl-1-naphthyl)-6,8-dimethoxy-1,2,3-trimethyl-1,2,3,4-tetrahydroisoquinoline [(+)-O-methylancstrocline]	Alkaloid
72	38.22	3.39	$C_{29}H_{50}O_4$	462	à-Tocospiro B	à-Tocospiro B
73	38.78	0.16	$C_{29}H_{50}O$	414	Stigmast-5-en-3-plL, (3á,24S)	Steroid
75	38.84	0.17	$C_{32}H_{54}O_4$	602	7,8-Epoxylanostan-11-ol, 3-acetoxy	Steroid

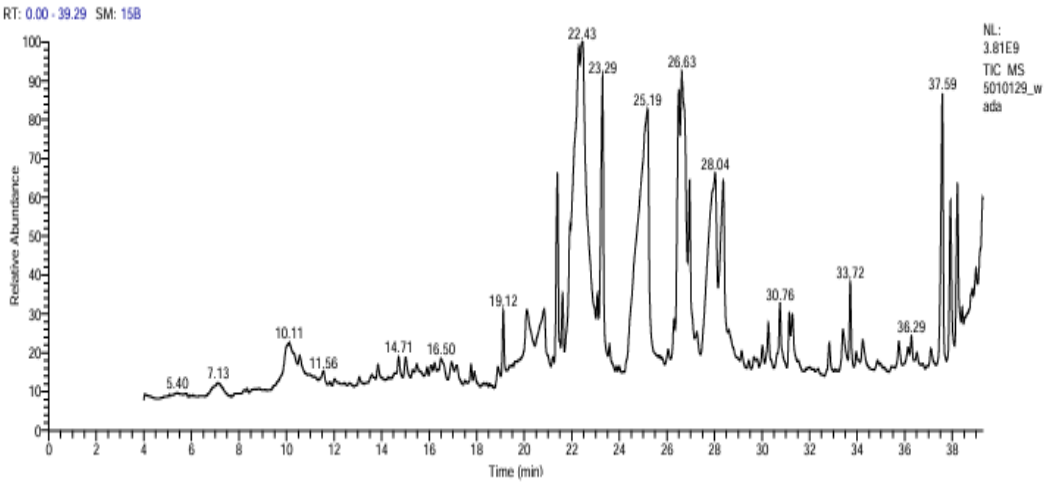

Figure 5. GC–MS chromatogram of *W. calenlulacea* leaves extract.

3.B. Antioxidant activity of *M. leucadendra, C. arvensis,* and *W. calenlulacea* leaves extracts

The antioxidant potential of *M. leucadendra*, *C. arvensis*, and *W. calenlulacea* leaves extracts was measured using a series of in vitro assays, including ABTS, DPPH, ORAC, FRAP, and metal chelation activity. The IC_{50} value was frequently used to assess the scavenging activity of *M. leucadendra*, *C. arvensis,* and *W. calenlulacea* extracts; the highest free radical scavenging activity is indicated by the lowest IC_{50} value.

The results demonstrate that *M. leucadendra* extract exhibits significant antioxidant properties, with varying degrees of efficacy across different assays. The findings suggest that *M. leucadendra* extract has the potential to mitigate oxidative stress, which is implicated in various chronic diseases, including cancer, cardiovascular disorders, and neurodegenerative conditions.

Based on the results of *M. leucadendra* extract, various tests were conducted to assess its antioxidant properties, which are crucial for understanding its potential anticancer benefits as illustrated in (**Table 6**):

Table 6. IC_{50} and Antioxidant Activity values of *M. leucadendra, C. arvensis,* and *W. calenlulacea leaves extracts* and positive controls.

	Melaleuca leucadendra **(IC_{50} or Activity)**	***Convolvulus arvensis*** **(IC_{50} or Activity)**	***Wedelia calenlulacea*** **(IC_{50} or Activity)**	**Positive Control (IC_{50} or Activity)**
ORAC Assay (µM Teq/mg) (Antioxidant activity)	4721.0 ± 433.18	1474.29± 84.99	796.05 ± 70.35	N/A
FRAP Assay (µM Teq/mg) (Antioxidant activity)	419.65 ± 30.53	61.67 ± 4.23	50.78 ± 2.84	N/A
ABTS Scavenging Activity (IC_{50}) µg/ml	14.84 ± 1.02	115.6 ± 2.78	N/A	Trolox: 22.05 ± 1.21 µM
DPPH Scavenging Activity (IC_{50}) µg/ml	39.42 ± 5.99	342.1 ± 3.99	404.7± 4.04	Trolox: 24.42 ± 0.87 µM
Metal Chelation Activity (IC_{50}) µg/ml	273.6 ± 5.41	526.9 ± 32.84	338.0 ± 12.84	EDTA: 20.18 ± 1.54 µM

i. Oxygen radical absorbance capacity (ORAC)

ORAC test measures the extract's ability to neutralize oxygen radicals, which is a common type of reactive oxygen species (ROS) involved in oxidative stress and cancer development. The ORAC assay demonstrated a high antioxidant activity of *M. leucadendra* extract, with a value of 4721.0 µM Trolox Equivalent (TE)/mg extract. However, the antioxidant activity of *C. arvensis* and *W. calenlulacea* ***leaves*** was lower than that of *M. leucadendra* extract, they were 1474.29 and 796.05 µM Trolox Equivalent (TE)/mg extract, respectively **(Table 6).** This indicates the strongest antioxidant activity of *M. leucadendra* extract among *C. arvensis* and *W. calenlulacea* extracts. This indicates a strong capacity to scavenge free radicals, which are highly reactive and contribute to oxidative damage in biological systems. On the other hand, the scavenging activity of *M. leucadendra* extract is higher than that of *C. arvensis* and *W. calenlulacea* extracts. This indicates a strong capacity to scavenge free radicals, which are highly reactive and contribute to oxidative damage in biological systems.

ii. Ferric reducing antioxidant power (FRAP)

The results from the FRAP assay indicated that the reducing power of *M. leucadendra* extract is 419.65 µM TE/mg

extract, while the reducing power of *C. arvensis* and *W. calenlulacea* extracts are 61.67 and 50.78 µM TE/mg extracts, respectively **(Table 6).** These results reflect that *M. leucadendra* extract capacity can donate electrons to convert ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), a crucial mechanism of antioxidant action. Because of *M. leucadendra* extract high FRAP value, *M. leucadendra* extract may have anticancer benefits by efficiently donating electrons to neutralize reactive species and reduce oxidative damage.

iii. ABTS radical scavenging activity

This test measures the ability of the extract to scavenge ABTS radicals. The IC_{50} value, which is the concentration required to inhibit 50% of radical activity, is an indicator of potency; a lower IC_{50} value implies higher antioxidant strength. The *M. leucadendra* extract exhibited an IC_{50} value of 14.84 µg/mL, significantly lower than that of Trolox (22.05 µM), indicating superior ABTS radical scavenging activity. This suggests that *M. leucadendra* extract has a strong capacity to neutralize ABTS radicals, which are commonly used to assess antioxidant potential. The *W. calenlulacea* IC_{50} isn't applicable while *C. arvensis* extract exhibited an IC_{50} value of 115.6 µg/mL, significantly higher than that of Trolox (22.05 µM), indicating a decrease in ABTS radical scavenging activity **(Table 6, Figure 6)**.

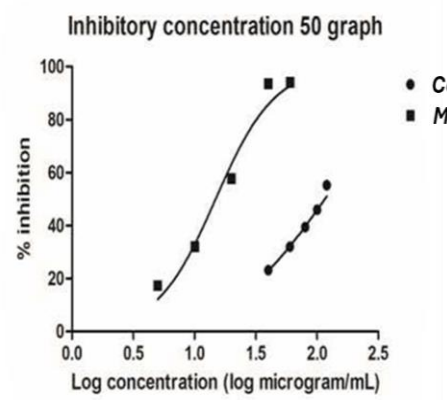

Figure 6. IC50 of *M. leucadendra* and *C. arvensis (CA)* leaves extracts from the ABTS method.

iv. DPPH radical scavenging activity

DPPH assay measures the free radical scavenging activity of the extract. A lower DPPH value represents a higher antioxidant activity since it indicates the extract's ability to donate electrons to neutralize free radicals. It revealed an IC_{50} value of 39.42 µg/mL for *M. leucadendra* extract, higher than that of Trolox (24.42 µM) indicating moderate free radical scavenging activity, suggesting that *M. leucadendra* extract can donate hydrogen atoms to neutralize DPPH radicals, although less effectively than Trolox. However, the IC_{50} values of 342.1 and 404.7 µg/mL for *C. arvensis* and *W. calenlulacea* leaves extracts, are significantly higher than that of Trolox (24.42 µM) (**Figure 7**) indicating very low free radical scavenging activity, suggesting that *C. arvensis* and *W. calenlulacea* extracts have very little donate hydrogen atoms to neutralize DPPH radicals **(Table 6, Figure 8)**.

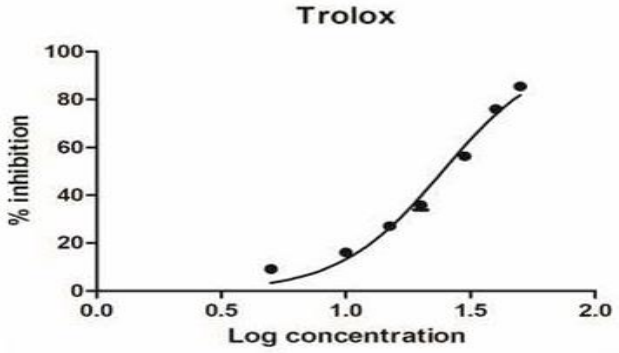

Figure 7. Calibration curve for TROLOX; used for DPPH method.

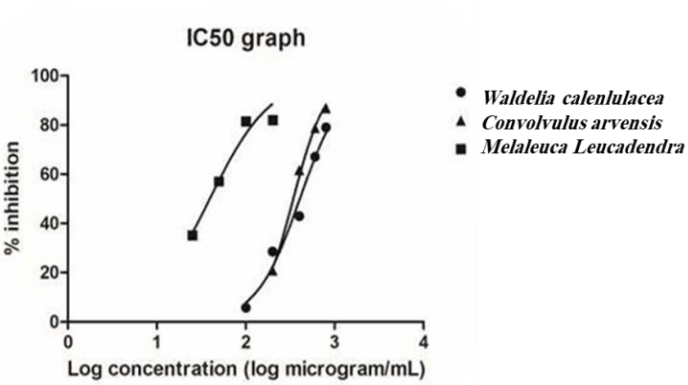

Figure 8. IC_{50} of *M. leucadendra*, *C. arvensis*, and *W. calenlulacea* leaves extracts of the DPPH method.

v. Metal chelation activity

Transition metals, like iron and copper, may catalyze the Fenton reaction. They have a major role in the generation of toxic oxygen radicals and cause oxidative stress and DNA damage linked to the development of cancer in living organisms. The production of these radicals can damage virtually all types of macromolecules and can lead to lipid peroxidation, protein modification, and DNA damage. Metal chelation is essential for binding and sequestering these metal ions. The metal ion chelation activity of *M. leucadendra* extract showed an IC_{50} value of 273.6 µg/mL, which was significantly higher than that of EDTA (20.18 µM). *M. leucadendra* extract is less effective than EDTA, although having a moderate level of metal chelation activity. However, by reducing the generation of ROS triggered by metals, this action could still add to its overall antioxidant effect. On the other hand, *C. arvensis* and *W. calenlulacea* extracts showed IC_{50} values of 338.0 and 526.9 µg/mL were more significant than that of EDTA (20.18 µM), respectively. *C. arvensis* and *W. calenlulacea* extracts are the least effective than EDTA and have weak levels of metal chelation activity **(Table 6, Figure 9)**.

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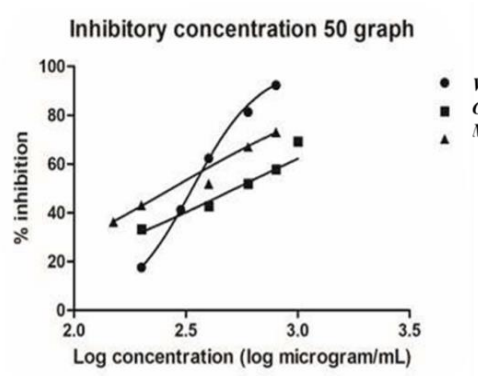

Figure. 9. IC_{50} of *M. leucadendra*, *C. arvensis*, and *W. calenlulacea* leaves extracts of Metal Chelation Activity assay.

DISCUSSION

This study investigated the phytochemical constituents and antioxidant properties of the methanolic extract of three plants, *Melaleuca leucadendra (ML), Convolvulus arvensis (CA),* and *Wedelia calendulaaceae (WC).*

Analysis of the phytochemicals in the leaves methanolic extracts of the three plants showed the presence of some bioactive compounds including terpenoids, steroids, tannins, saponins, and alkaloids. Previous studies reported the occurrence of rich phytochemical composition of medicinal plants (Cowan, 1999; Hostettmann & Marston, 2002). The presence of these bioactive compounds in the extracts of the three plants indicates that these compounds may be responsible for the biological activities of these plants such as antioxidant, anti-inflammatory, and/or included anticancer effects (Surh, 2003; Scalbert et al., 2005).

In the present study, HPLC analysis of the three methanolic extracts confirmed the presence of several phenolic and flavonoid components in the *M. leucadendra* extract are characterized by the presence of natural products of rutin, gallic acid, quercetin, and kaempferol. It has been shown that these compounds **(Table 2b)** have potent antioxidant activities, and they can scavenge free radicals (Rice-Evans et al., 1996; Pietta, 2000). *In vivo* and *in vitro* studies of gallic acid, which is a simple phenolic compound has been extensively studied for its antioxidant (Badhani et al., 2015), anti-inflammatory (Wu et al., 2022), and anticancer activities (Subramaniana et al., 2015). Many studies have demonstrated the ability of gallic acid to scavenge free radicals, inhibition of lipid oxidation (Sanchez-Morenoa et al., 1999), the therapeutic potentials of oxidative damage diseases and modulating various cellular pathways involved in inflammation and cancer development (Gao et al., 2019).

Rutin, quercetin, and kaempferol are flavonoids that play roles in the protection against oxidative stress, inflammation, and cancer (Middleton et al., 2000; Andersen & Markham, 2006). **Table 2b** shows the high amounts of those compounds in *M. leucadendra* extract indicate their roles in the biological activity of the plant.

The HPLC analysis of the extract from *Convolvulus arvensis* shows high amounts of caffeic acid, apigenin, and rutin **(Table 2c)**. Caffeic acid is known for its antioxidant, and anti-inflammatory effects (Scalbert, 1991), while apigenin has demonstrated protective effects against oxidative stress, inflammation, and cancer through a few mechanisms that include cell cycle arrest and apoptosis (Shukla & Gupta, 2010). The occurrence of rutin in *M. leucadendra* and *C. arvensis* highlights its effects as an important bioactive compound. Rutin, quercetin, kaempferol, gallic acid, apigenin, and caffeic acid are not detected by HPLC analysis in *W. calenlulacea* methanolic extract **(Table 2a)**, suggests that other compounds participate in different mechanisms of action for the therapeutic effects of *W. calenlulacea*. This plant has been reported to show hepatoprotective, antibacterial, cytotoxicity and neuroprotective, and cerebroprotective activities; possess antiosteoporotic activity, and found to be a remarkable chemopreventive agent (Lisa et al., 2014). Triterpenoids principle of *W. calendulacea* attenuated diethynitrosamine-induced hepatocellular carcinoma via down-regulating oxidative stress, inflammation and pathology via NF-kB pathway (Verma et al., 2018).

GC-MS analysis showed that the methanolic extracts of leaves of *M. leucadendra, C. arvensis,* and *W. calendulaaceae* contain some volatile and semi-volatile compounds that can contribute to the therapeutic effects of these plants. The GC-MS profile of the extract from the *M. leucadendra* indicates the presence of fatty acid esters, aziridine derivatives, and other bioactive compounds. It has been shown that fatty acid esters exert antimicrobial and anti-inflammatory effects (Kabara et al., 1972; Valgimigli & Gabbanini, 2015), while aziridine derivatives are recognized for their anticancer effects, which possibly can be used from their function (Khan et al., 2017, Salehi et al., 2019). Therefore, the GC-MS analysis supports the reported therapeutic actions of *M. leucadendra* extract as antibacterial, anti-inflammatory, and anticancer activities.

The GC-MS analysis of the methanolic extract from *C. arvensis* revealed the presence of fatty acid esters, monoterpenoids, steroids, and other bioactive compounds. Monoterpenoids are volatile compounds that exert antibacterial and anti-inflammatory effects (Bakkali et al., 2008). Some steroids can function as anti-inflammatory agents (Cutler, 2000). The presence of these compounds in the extract of *C. arvensis* provides a scientific basis for their antibacterial and anti-inflammatory activities (Sharifi-Rad et al., 2017). Additional research is required to understand the details of their contribution to the therapeutic effects of the plant.

The GC-MS analysis of *W. calenlulacea* extract showed the occurrence of fatty acid esters, sesquiterpenoids, steroids, and other bioactive compounds. Sesquiterpenoids represent a secondary metabolism of terpenoids with antibacterial and anti-inflammatory activities (Chadwick et al., 2013). The presence of steroids in *C. arvensis* supports the results which obtained in *W. calenlulacea*. It has been shown that *W. calenlulacea* has antibacterial and anti-inflammatory activities (Govindappa et al., 2013). The presence of fatty acid esters in the methanolic extracts of the three plant provides a basis for suggesting that these compounds engage

in their activities such as antibacterial and anti-inflammatory effects (Kabara et al., 1972, Valgimigli & Gabbanini, 2015).

This study investigated the antioxidant capacity of the methanolic extracts of leaves of *M. leucadendra*, *C. arvensis*, and *W. calendulaaceae* using multiple *in vitro* assays. Our data indicated that *M. leucadendra* exhibited the highest antioxidant capacity may be due to the highest amounts of total phenolic, flavonoid, and saponins among the three plants extracts.

The relatively high antioxidant activity of the *M. leucadendra* extract among the two other plants, which are *C. arvensis* and *W. calenlulacea* **(Table 6)** reported in this study indicated its significant ability to combat oxidative stress. Oxidative stress is characterized by an imbalance between the production of reactive oxygen species and the ability to neutralize them and which is a crucial factor in the development of some diseases such as cancer, and cardiovascular disorders as has been previously mentioned (Lobo et al., 2010; Pham-Huy et al., 2008). Earlier studies exhibited that the remarkable antioxidant properties of *M. leucadendra* extract indicate its therapeutic activities for the protection from some diseases.

The ORAC assay measures the ability of antioxidants to neutralize peroxyl radicals, a predominant type of oxygen radical involved in lipid peroxidation and DNA damage, both of which are critical events in cancer development and aging (Tudek et al., 2017). In This study, the higher ORAC value observed for the *M. leucadendra* extract indicates its stronger ability to scavenge these biologically relevant oxygen radicals compared to the other two plants extracts. This potent free radical scavenging activity highlights the ability of the *M. leucadendra* extract to protect biological systems from oxidative damage.

Another assay, the FRAP assesses the ability of antioxidants to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), a major mechanism of antioxidant action involving electron donation (Benzie & Strain, 1996). The high FRAP value exhibited by the *M. leucadendra* extract appeared to indicate its strong reducing power and its ability to neutralize free radicals by donating electrons. This electron-donating ability may be particularly important in interrupting radical chain reactions and preventing oxidative damage to biomolecules, which may contribute to its anticancer benefits through the neutralization of reactive species (Halliwell & Gutteridge, 2015).

In addition, the ABTS assay is used for assessing total antioxidant activity by measuring the scavenging of ABTS-stabilized radicals (Re et al., 1999). The IC_{50} value, which represents the extract concentration required to inhibit 50% of the radical activity, is inversely proportional to antioxidant potency. In this study, the lowest IC_{50} value observed in the *M. leucadendra* extract boosts its strong ability to neutralize free radicals, supporting its significant antioxidant potential.

The DPPH assay is also a common method for assessing free radical scavenging activity by measuring the reduction of the DPPH-stabilized radical by the antioxidant (Blois, 1958). The lower DPPH value of the *M. leucadendra* extract indicates higher antioxidant activity, suggesting that it is more effective at donating electrons or hydrogen atoms to stabilize the DPPH radical. However, the relatively lower DPPH activity compared to ORAC and ABTS, coupled with its lower metal chelating activity, suggests that the antioxidant mechanisms of the *M. leucadendra* extract may be more effective against certain types of radicals or through mechanisms other than metal chelation.

The correlation between the highest antioxidant capacity of the *M. leucadendra* methanolic extract **(Table 6)** and the highest content of phenolics, flavonoids, and saponins **(Table 3, Figure 3)** suggests that these phytochemicals play major roles in the plant biological effects. Phenolics and flavonoids are potent antioxidant agents, often attributed to their ability to scavenge free radicals, chelate metal ions, and modulate enzyme activities (Arts et al., 2001; Scalbert et al., 2005). Saponins have been also shown to have antioxidant activity through various mechanisms (Manzocco et al., 2001). The synergistic or additive effects of these phytochemicals in the *M. leucadendra* extract can contribute to its potent antioxidant activity.

CONCLUSION

HPLC analysis provides an overview of the bioactive compounds that occur in the leaves extracts of *M. leucadendra*, *C. arvensis*, and *W. calenlulacea.* The high content of therapeutic antioxidant phenolics and flavonoids in *M. leucadendra* and *C. arvensis* supports their traditional uses and health benefits.

GC-MS analysis provides information on the volatile and semi-volatile bioactive compounds that occur in the methanolic leaves extracts of the three studied plants. The presence of fatty acid esters, with aziridine derivatives, monoterpenoids, and sesquiterpenoids, supports the reported therapeutic activities of *M. leucadendra*, *C. arvensis*, and *W. calenlulacea*, respectively.

This study demonstrates that the methanolic extract of *Melaleuca leucadendra* has potent antioxidant properties, which is attributed to its high content of phenolic compounds, flavonoids, and saponins. This suggests that *Melaleuca leucadendra* extract may mitigate oxidative stress and can be used to prevent or treat some chronic diseases.

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