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RESEARCH ARTICLE

Seasonal variation in chemical compositions and biological activities of essential oils extracted from *Ageratum conyzoides* L. - grown in Western Ghats region-South India

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Abstract

The study aimed to characterize the influence of seasonal variation on the chemical composition and biological activities of essential oils extracted from the leaves of *Ageratum conyzoides* L. (*A. conyzoides*). Essential oils were extracted in summer (May), winter (January), and autumn (September) by hydrodistillation. GC/MS identified the volatiles compounds. The major compounds present in the essential oils across the three different seasons were Precocene II (29.44, 39.8 and 43.90%), Precocene I (32.17, 22.8 and 20.14%), and Caryophyllene oxide (9.09, 11.50 and 10.2%), followed by Carvone Hydrate (4.39, 4.65 and 3.65%) and Germacrene D (2.42, 2.25 and 2.76%). Biological activities such as in-vitro antioxidant, anti-cancer, and anti-bacterial activities were evaluated. The essential oil from *A. conyzoides* leaves exhibited strong antioxidant activity with IC_{50} values of 34.17 µg/mL for DPPH and 33.47 µg/mL for ABTS assays in summer; 99.92 µg/mL for DPPH and 26.40 µg/ mL for ABTS in winter; and 96.47 µg/mL for DPPH and 78.59 µg/mL for ABTS in autumn. The essential oil showed significant anticancer activity against the MDA-MB-231 breast cancer cell line with IC_{50} value of 122±0.05 µg/mL in summer, 86.50±0.05 µg/mL in winter, and 148.38 ± 1.15 µg/mL in autumn, compared to and Etoposide at 30 µg/mL. The antibacterial activity against two gram-positive and four Gram-negative bacteria is moderate to good.

Keywords

Ageratum conyzoides L.; essential oil; GC–MS; *in-vitro* antioxidant activity; *in-vitro* anti-cancer; *in-vitro* antibacterial activity

Introduction

Ageratum conyzoides L. is an annual herb that grows wild in tropical and subtropical zones. It has a long history of traditional medicinal uses worldwide, including treatments for ulcers, as a purgative, febrifuge, for ophthalmia, colic, headaches, dyspnoea, and as an anti-asthmatic, anti-spasmodic, analgesic, anti-diarrhoeic, anti-inflammatory, and for gynecological diseases. Numerous studies on the chemical composition and biological activity of the essential oil of *A. conyzoides* from various countries have been reviewed (1). The high contents of chromenes, specifically Precocene I (7-methoxy-2, 2-dimethyl chromene) and Precocene II (6, 7-dimethoxy-2, 2-dimethyl chromene or ageratochromene), has been reported inall specimens studied. In India, an Ayurvedic study found that

the plant's root is useful in treating fever and possesses anti-helminthic and anti-dysenteric properties (2). Further investigation revealed that the essential oil contains benzopyrans, flavonoids, and terpenoids (3-10). The chromenes, including Precocene I and II, found in *Ageratum* species, (10-13) exhibit significant biological activity against various insect species (13-16). Additionally, the plant shows morphological flower variations, ranging from white to purple, linked to different chemotypes (17).

In a review of the available literature, no publications were found on the seasonal variation in the chemical composition of *A. conyzoides*, indicating a substantial gap in this field. Since this species is neither endemic nor endangered, there is anticipated interest in its essential oil. Furthermore, no studies have been reported in the Western Ghats region. Therefore, the current investigation aims to evaluate the seasonal variation in the chemical composition and biological activities of *Ageratum conyzoides* L essential oil (ACEO).

Materials and Methods

Plant material

Fresh leaves of *A. conyzoides* were collected from Pallanadu Marayoor Idukki District, Kerala (Longitude 77.140018, latitudes 10.23495) during May, January, and September 2021. The plant was identified by the Botanical Survey of India, Coimbatore (Voucher No.BSI/SRC/5/23/2022/Tech/643).

Extraction procedure

The collected leaves of *A. conyzoides* were cut into pieces and distilled using a Clevenger-type apparatus. The leaves were soaked in water and boiled for 3-4 hours, yielding a yellowish-green essential oil with a strong odour. The volatile oil was collected in a flask, and separation from the aqueous layer was performed using a separating funnel with petroleum ether as the non-polar solvent. The essential oil was then dried over anhydrous sodium sulphate to remove moisture and stored at -4°C until tested. This extraction process was repeated several times to produce the required volume of oil for further testing.

Characterization of the essential oil by GC-MS analysis

GC-MS analysis of ACEO

The volatile constituents in the essential oils were identified using a gas chromatograph (Agilent 7890A) equipped with an HP-5 MS 5% phenyl methyl siloxane capillary column (30.00 m × 0.25 mm, 0.25 µm film thickness). Helium (99%) was used as the carrier gas at a flow rate of 2mL/min, with0.1 µL samples injected in split mode at a 60:1 ratio. The oven temperature was initially set at 60°C for 3 min and then raised at a rate of 4°C/min to 260° C. The injector and detector temperatures were set at 250°C and 280°C, respectively. The peak area percentage was used for quantitative analysis. The gas chromatograph was coupled with an Agilent 5975C mass selective detector. The EI-MS parameters were as follows: ionization voltage of 70eV and ion source temperature of 200^{0}C. Oil components were identified by comparison of their mass spectral fragmentation patterns with the WILLEY/ Chem Station data system (18).

In-vitro anti-oxidant activity of ACEO

Free radical scavenging activity (DPPH)

The ability of ACEO to scavenge free radicals was investigated using the 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) method. Experiments were conducted with a freshly prepared 2.9 mL DPPH (0.1mM) solution in methanol, to which different concentrations of ACEO (10, 25, 50, 75, and 100 µg/mL) were added. The mixtures were kept at 25°C in a dark area for 20-30 minutes, and absorbance was measured at 517 nm using a UV-visible spectrophotometer after 30 minutes. A control experiment, without ACEO, was conducted using the same approach. Ascorbic acid solution was used as a standard. Radical scavenging activity (RSA) was calculated by the following equation:

$$\% \text{ RSA} = (Abs\ control - Abs\ sample)/(Abs\ control) \times 100 \quad (1)$$

Where RSA stands for Radical scavenging activity; Abs control represents the absorbance of DPPH radical + ethanol; and Abs sample represents the absorbance of DPPH radical + sample essential oil (19).

ABTS assay of ACEO

The scavenging activity of the essential oil from *A. conyzoides* leaves against the $ABTS^{\cdot+}$ radical cation was determined. Various concentrations of ACEO (10, 25, 50, 75, and 100 µg/ mL) were mixed with the required amount of ABTS solution. The absorbance of the sample was measured at 734 nm after an hour of incubation at 25°C. The $ABTs^{\cdot+}$ scavenging activity was calculated by the following formula:

$$\% \text{ A} = (Abs\ control - Abs\ sample) / (Abs\ control) \times 100 \quad (2)$$

Where Abs control is the absorbance of the control, and Abs sample is the absorbance ACEO (20).

In-vitro anti-cancer activity of ACEO

The anticancer activity of ACEO was analyzed using the MTT assay in the MDA-MB-231 breast cancer cell line, which was purchased from the National Centre for Cell Science, Pune, India. MDA-MB-231 (5×10^4) cells were cultured in a 96-well plate with complete Dulbecco's Modified Eagle Medium (DMEM) medium and incubated for 24 h at 37°C in 5% CO_2. After 24 h of exponential growth, the cells were exposed to different concentrations (0-200µg/ ml) of the compound, with vehicle control (0.05% DMSO) serving as the negative control and Etoposide as the positive control. After 24 h of treatment, 10 µL of 4,5-dimethylthiazol-2-yl-2,5-diphenyltetrazolium bromide (MTT) (5 mg/ ml) was added to each well and incubated for 4 h at 37°C. The resulting purple formazan crystals were solubilized in 150 µL of DMSO, and the colour development was recorded at a wavelength of 570 nm (21).

In-vitro antibacterial activity of ACEO

Clinical isolates of bacterial strains were obtained from the KMCH hospitals, in Coimbatore. Bacteria were cultured

overnight at 37°C on nutrient agar (NA, Oxoid). ACEO was tested against the following microorganisms: Gram-positive: *Staphylococcus aureus, Bacillus subtilis.* Gram-negative*: Salmonella paratyphi A, Escherichia coli, Klebsiella pneumonia, pseudomonas aeruginosa.* The antimicrobial present in the ACEO diffuses into the medium and interacts with the test organisms on a freshly seeded plate. The diameter of the inhibition zone is measured in millimeters.

Petri plates containing 20mL Muller Hinton medium were seeded with a 24-hour culture of bacterial strains. Wells were cut, and 20 µl of ACEO was added. The plates were incubated at 37°C for 24 hours. Antibacterial activity was assessed by measuring the diameter of the inhibition zone around the well (NCCLS, 1993). Chloramphenicol disc served as a positive control, and DMSO (dimethyl sulfoxide) was a negative control (22).

Determination of minimum inhibitory concentration of ACEO

Dilution susceptibility testing methods are used to determine the minimal concentration of an antimicrobial needed to inhibit or kill a microorganism. This is achieved by diluting the antimicrobial in either agar or broth media. Antimicrobials are tested in $\log^2$ serial dilutions (two-fold). The minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. For this procedure, 100 µl of medium (nutrient broth for bacteria) was distributed from the 1st to the 6th well of a 96-well polypropylene micro titer plate. The antimicrobial (ranging from100µg/ml to1.25µg/ml) was added to the 2nd well of each row, and then 100 µl serial dilutions were performed from the 2nd to the 6th well. The 11th well served as a growth control without the sample. Next,100 µl of pathogenic strains were added to all the wells. The plates were incubated at 37°C for 24 h and then read at 578 nm using an ELISA reader (23).

Statistical analysis

Every study was conducted at least three times. The findings are presented as Mean ± Standard deviation. IC_{50} values were calculated using the sigmoidal dose-response formula in Origin Pro 8.5 software.

Results and Discussion

GC–MS analysis of essential oils extracted from the leaves of *A. conyzoides* identified of 61 different compounds, representing 96-98.5% of the oil, although some minor compounds were not identified in different seasons. The main components present in essential oils were Precocene II (29.44, 39.8 and 43.90%), Precocene I (32.17, 22.8 and 20.14%) and Caryophyllene oxide (9.09, 11.50 and 10.2%) followed by Carvone Hydrate (4.39, 4.65 and 3.65%) and Germacrene D (2.42, 2.25 and 2.76%). In all three seasons, the majority of compounds belonged to the Precocene I, Precocene II, and Caryophyllene oxide chemotype

The ACEO contained higher levels of chromenes (Precocenes) Precocene I and II (61.61, 62.6 and 64.09%), compared to monoterpenoids and sesquiterpenoids. Structurally related monoterpenes made up 11 out of 61 constituents, accounting for 18.03% of the total oil, while sesquiterpenoids comprised 19 out of 61 constituents or 31.14% of the oil. Oxygenated compounds included 20 out of 61 constituents, making up 32.8% of the oil. Aromatic hydrocarbons were 2 out of 61 constituents (3.27%), phenyl propene was 1 out of 61 constituents (1.16 %), and the remaining hydrocarbons, 6 out of 61 constituents, accounted for 9.8% of the oil.

The principal constituents of *A. conyzoides* essential oil from North West Karnataka were Precocene I, II and Caryophyllene (24). Recent comprehensive studies on the chemical variability of hydrodistilled essential oils from various wild-growing and cultivated populations of *A. conyzoides* from the Indian Ocean, China, Nigeria, and Pakistan, as well as supercritical fluid extracts of various *A. conyzoides* species confirmed that high concentrations of Precocene I, II and Caryophyllene are the main chemo taxonomic attributes of *A. conyzoides* essential oil (25-28). The main constituent of these oils was precocene I (65.97 to 78.42 %), followed by E-caryophyllene (6.04 to 12.16 %), comprising an average of 79.87 % of the composition throughout the year (29). The leaf essential oil of plants from India was dominated by Chromenes and sesquiterpene derivatives. This is the first report on the leaf essential oil of *A. conyzoides* L. from the Western Ghats region.

However, although Precocene I, II, and Caryophyllene are present in all samples, their amounts vary from plant to plant. Quantitatively, a higher percentage of Precocene II is found in the ACEO of plants from the Western Ghats region. This Precocene II component is present only in trace amounts in plants from the Ivory Coast and Western Fiji, using flower and stem oils. It is completely absent in essential oils from Nigeria and northern Brazil. The difference in the chemical composition of ACEO can be attributed to climatic variations specific to each location, highlighting the importance of geo-ecological factors in the production of plant metabolites (30).

In the present study, the DPPH radical scavenging activity of *A. conyzoides* essential oil was tested for the concentration from 100, 200, 300, 400, and 500 µg/mL, with the results shown in Table 2. The essential oil exhibited DPPH radical scavenging activity in a concentration-dependent manner, comparable to the commercial standard ascorbic acid. The scavenging activities were observed as follows: Summer season 28.4%, 56.7%, 58.2%, 71.5%, 71.8%; winter season 8.5%, 9.9%, 21.4%, 28.6%, 44.3%; and autumn season 42.9, 49.1, 53.9, 58.1, 61.7%. For comparison, the standard ascorbic acid values were 80.85%, 87.23%, 89.36%, 93.61%, and 97.87%. These results indicate that *A. conyzoides* essential oil can act as an antioxidant, effectively scavenging free radicals and reactive oxygen species.

The essential oil was also evaluated for its anti-oxidative capacities by examining its $^{-}$OH scavenging effects using DPPH and ABTS radicals at concentrations of

Table 1. Seasonal variation in the Chemical composition of the essential oil of *A. conyzoides* leaves

Sr. No.	Compound	RI	% Relative content		
			Summer	Winter	Autumn
1	Heptanol<2->	899	2.72	0.07	-
2	Santolinatriene	909	1.62	-	-
3	Pyrazine<2.6-dimethyl->	913	-	0.1	-
4	Thujene<α->	933	0.53	-	0.8
5	Cymene <p->	1026	1.31	0.14	-
6	Bornyl acetate	1045	1.02	-	0.42
7	Terpinene<y->	1060	-	0.23	-
8	Artemisia ketone	1065	0.46	-	0.1
9	Acetophenone	1067	-	0.42	-
10	Mentha-3.8-diene <p->	1075	-	0.48	0.62
11	Linalool	1086	0.19	-	**1.06**
12	Pinene epoxide	1094	-	-	0.23
13	Cymene <meta->	1086	-	0.11	-
14	Methyl benzoate	1091	-	0.11	-
15	Epoxymyrcene<6,7->	1093	1.53	1.33	0.3
16	Camphene	1072	0.14	-	-
17	Copaene	1075	0.37	-	-
18	Caryophylleneoxide	1090	9.09	11.5	10.2
19	Hexyl propanoate	1102	-	2.76	-
20	Octadienal<2.2-dimethyl-3,4->	1104	0.08	3.1	-
21	1,6-Cyclodecadiene	1105	2.73	-	0.5
22	alpha.-Cubebene	1108	3.3	-	0.1
23	Isophorone	1123	0.44	0.05	-
24	Menth-2-en-l-ol <cis-p->	1124	-	0.38	-
25	Undecyne<1->	1126	0.2	0.17	0.21
26	Ocimene<allo->	1134	0.16	0.3	-
27	Pyrazine<3-methyl-2-isobutyl->	1139	0.21	-	-
28	Nopinone	1143	0.49	-	-
29	Hexenylisobutanoate «3Z)->	1147	-	0.72	-
30	Menthone	1155	0.61	-	0.7
31	Thujanol<neo-3->	1156	0.07	0.26	-
32	Artemisyl acetate	1175	0.26	0.26	-
33	Rosefuran epoxide	1179	0.44	0.46	0.21
34	Cryptone	1188	0.18	0.11	-
35	Linalool acetate <tetrahydro->	1236	0.17	0.37	-
36	β-Cubebene	1375	0.04	-	0.48
37	Carvone hydrate	1388	4.39	4.65	3.65
38	Methyl eugenol	1409	-	-	0.11
39	Cis-Carvyl propionate	1438	-	-	0.11
40	Humulene	1443	-	-	1.33
41	Precocene-I	1447	32.17	22.8	20.14
42	α-Caryophyllene	1450	-	-	0.63
43	Germacrene-D	1468	2.42	2.25	2.76
44	Muurolene	1474	-	-	3.1
45	Cadinene	1483	-	0.11	0.2
46	Cis-Guaiene	1492	-	-	1.25
47	Bisabolene	1503	-	1.08	1.31
48	Cadinene	1513	-	-	0.16
49	Cadina-1,4-diene	1538	-	-	0.38
50	β-Sesquiphellandrene	1552	-	-	0.17
51	Spathulenol	1572	-	-	0.3
52	Globulol	1578	0.52	-	0.22
53	Caryophyllene	1589	-	0.95	0.78
54	Bisabola-2,10-diene 1,9-oxide	1592	-	-	0.15
55	Cadinol	1611	-	-	0.72
56	5-Guaiene-11-ol	1617	-	-	0.26
57	Eudesmol	1620	-	-	0.13
58	Cubenol	1630	-	-	0.12
59	Germacradiene-11-ol	1634	-	-	0.26
60	Precocene II	1658	29.44	39.8	43.9
61	Farnesol	1685	-	-	0.37
			97.42	96.69	98.44

Table 2. DPPH Scavenging Activity of ACEO–Summer, winter and autumn seasons

Treatments	Concentration (µg/mL)					IC_{50} (µg/mL)
	100	200	300	400	500	
Ascorbic acid	80.85	87.23	89.36	93.61	97.87	13.8
Summer (%)	28.4	56.7	58.2	71.5	71.8	34.17
Winter (%)	8.5	9.9	21.4	28.6	44.3	99.92
Autumn (%)	42.9	49.1	53.9	58.1	61.7	96.47

100, 200, 300,400and 500 µg/mL (Table 2 and 3). The essential oil demonstrated IC_{50}value as: summer: 34.17 µg/mL for DPPH and 33.47 µg/mL for ABTS; winter: 99.92 µg/mL for DPPH and 26.40 µg/mL for ABTS, and autumn: 96.47 µg/mL for DPPH and 78.59 µg/mL for ABTS. These results were compared with the standard ascorbic acid, which showed IC50 values of 13.8 µg/mL for DPPH and 14.4 µg/mL for ABTS.

Previous literature indicates that both the essential oil and methanol extract of *A. conyzoides* were assessed for their antioxidant activity. The methanol extract demonstrated the highest antioxidant activity in the FRAP and DPPH assay, while the essential oil exhibited greater lipid peroxidation inhibition compared to the ethanol extract (31). Further studies have shown that *A. conyzoides* positively affect the redox system in diabetic rats induced by streptozotocin, improving their glycaemic status (32).

The present study presents the anticancer potential of ACEO for the summer, winter, and autumn seasons, which are described in (Tables 4, 5 and 6 and Fig. 1, 2 and 3). The viability of MDA-MB-231 cancer cells was assessed after incubation with various (0-200 µg/mL) of the essential oil, alongside the commercial cancer drug Etoposide, using the MTT assay. The results demonstrated that the essential oil-induced significant cytotoxicity in a concentration-dependent manner. The essential oil showed the highest anticancer activity against the MDA-MB-231 (breast cancer) cell line, with IC_{50} values of 122±0.05 µg/mL in summer, 86.50±0.05 µg/mL in winter, and 148.38±1.15 µg/mL in autumn, compared to Etoposide with an IC50 value of 30 µg/mL. These results indicate that the essential oil of *A. conyzoides* leaves exhibited the best anticancer activity in the winter season compared to the other two seasons. The findings suggest that *A. conyzoides*

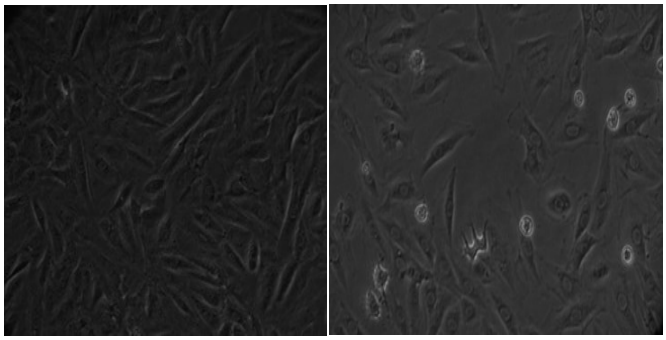

Fig 1. *In-vitro* anti-cancer activity of ACEO–summer (Lower anti-cancer activity)

Table 3. ABTS scavenging activity of ACEO– summer, winter and autumn

Treatments	Concentration (µg/mL)					IC_{50} (µg/mL)
	100	200	300	400	500	
Ascorbic acid	52.63	96.83	97.36	98.42	99.47	14.4
Summer (%)	60	67	74	82	83	33.47
Winter(%)	17	30	32	43	61	26.40
Autumn(%)	26.17	32.34	49.8	57.87	61.27	78.59

Table 4. *In-vitro* anti-cancer activity of ACEO - summer

ACEO		0	20	40	60	80	100	120	140	160	180	200
Test-1	0.05	1.411	1.325	1.196	1.032	0.871	0.766	0.743	0.551	0.403	0.241	0.066
Test-2	0.046	1.303	1.283	1.125	0.933	0.889	0.808	0.695	0.521	0.322	0.283	0.022
Test-3		1.357	1.304	1.161	0.983	0.880	0.787	0.719	0.536	0.363	0.262	0.044
% of cell viability		**0**	**4**	**14**	**28**	**35**	**42**	**47**	**61**	**73**	**81**	**97**

IC_{50} =122.55± 0.05 µg/mL

Table 5. *In-vitro* anti-cancer activity of ACEO - winter

ACEO		0	20	40	60	80	100	120	140	160	180	200
Test-1	0.05	1.497	1.284	1.115	0.957	0.759	0.584	0.453	0.287	0.161	0.067	0.037
Test-2	0.046	1.331	1.122	0.99	0.868	0.691	0.552	0.392	0.242	0.147	0.041	0.032
Test-3		1.414	1.203	1.053	0.913	0.725	0.568	0.423	0.2645	0.154	0.054	0.035
% of cell viability		**0**	**15**	**26**	**35**	**49**	**60**	**70**	**81**	**89**	**96**	**98**

IC_{50} =86.50± 0.05 µg/mL

Table 6. *In-vitro* anti-cancer activity of ACEO - autumn

ACEO		0	20	40	60	80	100	120	140	160	180	200
Test-1	0.05	1.411	1.325	1.196	1.032	0.871	0.766	0.743	0.551	0.263	0.141	0.066
Test-2	0.046	1.303	1.283	1.125	0.933	0.889	0.808	0.695	0.521	0.222	0.113	0.082
Test-3		1.357	1.304	1.161	0.983	0.880	0.787	0.719	0.536	0.243	0.127	0.074
% of cell viability		**0**	**4**	**14**	**28**	**35**	**42**	**47**	**61**	**82**	**91**	**95**

IC_{50}=148.38 ± 1.15 µg/mL

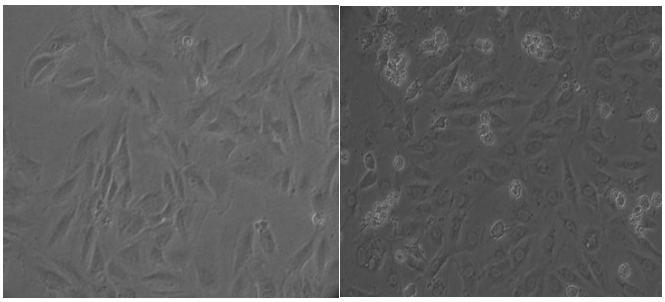

Fig 2. *In-vitro* anti-cancer activity of ACEO-winter (better anti-cancer activity)

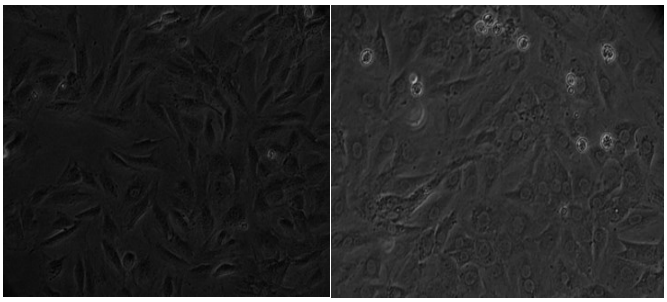

Fig 3. *In-vitro* anti-cancer activity of ACEO-autumn (Lowest anti-cancer

essential oil could be a potential source of alternative medicine for cancer treatment.

There are only limited results regarding the anticancer activity of *A. conyzoides* leaf extracts. Ethanol and petroleum ether extracts have been tested against the P-388, A-549, SGC-7901 and A-549, DU-145, and SGC-7901 cell lines. Similarly, ethyl acetate extract has been tested against the A-549, DU-145, SGC-7901, and P-388 cells, respectively (33).

The antibacterial activity of ACEO was tested against two gram-positive bacteria *Staphylococcus aureus, and Bacillus subtilis* and four Gram-negative bacteria *Salmonella paratyphi A, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa.* The agar-well diffusion method was employed with concentrations ranging from 25µg/ml to 100 µg/ml, as shown in Tables 7, 9 and 11. Gram-positive bacteria generally showed higher sensitivity to the essential oil compared to gram-negative bacteria. Significant antibacterial activity was observed for *S. aureus, E coli, K. pneumonia,* and *P. aeruginosa* across all

Table 7. *In-vitro* antibacterial activity of ACEO in summer

S. No	Zone of inhibition in mm							
	Concentration	***S. aureus***	***S.paratyphi* A**	***E. coli***	***K.pneumoniae***	***P.aeruginosa***	***B. subtilis***	**Chloramphenicol**
1	25	11.3±0.5	2.6±0.1	2.41±1	10.3±1.5	3.3±0.2	2.6±0.5	16.5±0.9
2	50	13.33±0.2	5.6±0.2	5.44±0.5	12.23±0.1	8.25±0.6	6.21±0.4	17.2±0.4
3	75	16.36±0.1	9.6±0.3	8.41±1	15.21±0.7	13.26±0.3	9.31±0.3	20.6±0.6
4	100	19.23±0.4	12.6±0.5	13.36±1	19.31±0.9	17.27±0.2	14.32±0.2	23.2±0.2

Table 8. Minimum Inhibitory Concentration of ACEO- summer

S. No	Organism	MIC µg/ml
1	*S. aureus*	50
2	*S. paratyphi* A	100
3	*E. coli*	100
4	*K. pneumonia*	100
5	*P. aeruginosa*	100
6	*B. subtillis*	100

Table 10. Minimum inhibitory concentration of ACEO -winter

S. No	Organism	MIC µg/ml
1	*S. aureus*	50
2	*S. paratyphi* A	100
3	*E. coli*	100
4	*K. pneumonia*	100
5	*P. aeruginosa*	100
6	*B. subtillis*	100

Table 9. *In-vitro* anti-bacterial activity of ACEO in winter

S. No	Zone of inhibition in mm							
	Concentration	***S. aureus***	***S. paratyphi* A**	***E. coli***	***K. pneumoniae***	***P. aeruginosa***	***B. subtilis***	**Chloram phenicol**
1	25	14.6±0.21	5±0.19	2.6±0.53	7.6±0.33	5±1	2.6±0.28	19.8±0.72
2	50	16.43±0.13	7.42±0.83	6.43±0.65	13.43±0.17	9.22±0.74	5.11±0.92	20.2±0.22
3	75	19.38±0.71	9.51±0.57	7.31±0.92	17.51±0.63	15.24±0.35	9.21±0.64	24.3±0.27
4	100	22.22±0.86	14.32±0.59	10.26±0.21	21.42±0.92	18.28±0.23	13.22±0.52	26.2±0.45

Table 11. *In-vitro* anti-bacterial activity of ACEO in autumn

S. No	Zone of inhibition in mm							
	Concentration	***S. aureus***	***S. paratyphi* A**	***E. coli***	***K.pneumoniae***	***P.***	***B. subtilis***	**Chloramphenicol**
1	25	12.15±1	10.25±1	4.5±0.83	6.5±0.43	2.5±0.24	6.51±0.1	17.2±0.33
2	50	14.53±0.91	14.22±0.45	6.41±0.38	12.33±0.61	6.22±0.72	9.32±0.3	28.6±0.54
3	75	18.28±0.72	16.31±0.22	7.33±1	17.31±0.8	9.24±0.44	12.31±0.3	22.9±0.67
4	100	23.26±0.81	19.42±0.61	11.25±1	21.42±0.53	12.28±0.36	15.23±0.77	27.3±0.45

Table 12. Minimum Inhibitory Concentration of ACEO -autumn

S. No	Organism	MIC µg/ml
1	*S. aureus*	50
2	*S. paratyphi* A	100
3	*E. coli*	100
4	*K. pneumonia*	50
5	*P. aeruginosa*	100
6	*B. subtillis*	50

concentrations. The remaining bacterial strains also exhibited moderate to good antibacterial activity at a tall tested concentration.

The bacteriostatic efficacy of ACEO was assessed using the broth dilution method, with serial dilutions ranging from 25, 50, 75, and 100 µg/mL. In both the summer and winter seasons *S. aureus* had a minimum inhibitory concentration of 50 µg/mL, while *S. paratyphi* A, *E. coli, K. pneumoniae*, *P. Aeruginosa,* and *B. subtilis* showed MIC values of 100 µg/mL. In the autumn season, *S. Aureus, K. pneumoniae,* and *B. Subtilis* had MIC values of 50 µg/mL, whereas *S. paratyphi* A, *E. Coli, and P. Aeruginosa* showed MIC values equal to 100 µg/mL. In the autumn season, *S. aureus*, *K. pneumoniae*, and *B. subtilis* had MIC values of 50 µg/mL, whereas *S. paratyphi* A, *E. coli*, and *P. aeruginosa* had MIC values of 100 µg/mL.

Furthermore, the anticancer and antibacterial activities of the essential oil were evaluated to determine its biological potential. The results revealed potent inhibitory cytotoxic effects of *A. conyzoides* leaf essential oil against cell lines. It is important to note that prominent constituents of the oil such as terpenes like Caryophyllene, and humulene, have demonstrated various biological activities, including anticancer and antibacterial activity (34).

The antibacterial activity of ACEO demonstrated that the strongest antibacterial effects were observed against *Staphylococcus aureus* and *Bacillus subtilis* in a disc diffusion assay (31). ACEO was tested against seven bacterial strains, with inhibition zones ranging from 6.7 to 12.7 mm and minimum inhibitory concentrations (MICs) between 64 and 256 µg/mL for strains sensitive to ACEO. Notably, ACEO exhibited moderate antibacterial activity against *Staphylococcus aureus* and *Enterococcus faecalis* (30).

Conclusion

This study investigated the chemical composition, in-vitro antioxidant, anticancer, and antimicrobial activities of essential oil extracted from the leaves of *Ageratum conyzoides* (ACEO) across three different seasons. The antioxidant activity of ACEO was found to be superior to that of standard ascorbic acid. The essential oil demonstrated significant anticancer activity against the MDA-MB-231 breast cancer cell line and showed promising results compared to the commercial drug Etoposide. These findings suggest that ACEO has potential as an alternative medicine for cancer treatment. Furthermore, ACEO exhibited notable antibacterial activity across all tested concentrations, which may be attributed to the high levels of Precocene I, Precocene II, and Caryophyllene oxide present in the oil. The study highlights the potential of ACEO as a natural source of antioxidants, antibacterial agents, and anticancer compounds. Among the three seasons, the essential oil harvested in winter provided the highest yield and demonstrated superior biological activities. Based on these results, winter is the optimal season for harvesting ACEO for both medicinal and commercial purposes. Further research is needed to elucidate the precise mechanisms underlying the biological activities of ACEO.

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Authors' contributions

Dr. PK helped participate in the sequence alignment and drafted the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: None

Ethical issues: None

References

1. Okunade AL. *Ageratum conyzoides* L. (Asteraceae)-A review. Fitoterapia. 2002; 73 (1): 1–16. http://dx.doi.org/10.1016/S0367-326X (01)00364-1.
2. Perumal Samy R, Igancimuthu S, Patric RD. Preliminary Screening of ethnomedicinal plants from India. J Ethnopharmacol. 1999; 66: 235-240.
3. Gonzalez AG, Aguiar ZE, Grillo TA, Luis JG, Rivera A, Calle J. Chromenes from *Ageratum conyzoides.* J. Phytochemistry.1991;

30:1137-1139. https://doi.org/10.1016/S0031-9422(00)95190-2.

4. Borthakur N, Baruah AKS, Bhagat SD. Search for Precocenes in *Ageratum conyzoides* Linn. Of north-east Índia. J. Ind. Chem. Soc. 1987; 64: 580-581.
5. Gonzalez AG, Aguiar ZE, Grillo TA, Luis JG, Rivera A, Calle J. Methoxyflavones from *Ageratum conyzoides*. Phytochemistry. 1991; 30:1269-1271.https://doi.org/10.1016/S0031-9422(00)95215-4
6. Vyas AV, Mulchandani NB. Polyoxygenated flavones from *Ageratum conyzoides*. Phytochemistry.1986; 25(11): 2625: 2627. https://doi.org/10.1016/S0031-9422(00)84523-9
7. Aalbersberg WGL, Singh Y. Essential oil of Fijian, *Ageratum conyzoides*.Flavour Fragr J. 1991; 6: 117-120.https://doi.org/10.1002/ffj.2730060204
8. Dung NX, Tho PTT, Dan NV, Leclercq PA. Chemical composition of the oil of *Ageratum conizoides* L. from Vietnam. J Essent Oil Res.1989; 1:135-136. https://doi.org/10.1080/10412905.1989.9697769
9. Mensah M, Sarpong K, Baser KHC, Ozek T. The essential oil of *Ageratum conizoides* L. from Ghana J Essent Oil Res. 1993; 5:113-115. https://doi.org/10.1080/10412905.1993.9698184.
10. Menut C, Lamaty G, Zollo PHA, Kuiate JR, Bessière JM. Aromatic plants of tropical central Africa. Part X Chemical composition of the essential oils of *Ageratum houstonianum* Mill. and *Ageratum conyzoides* L. from Cameroon. Flavour Fragr J. 1993; 8:1-4. https://doi.org/10.1002/ffj.2730080102 .
11. Quijano L, Calderon JS, Gonbmez GF, Soria IE, Rios T. Highly oxygenated flavonoids from *Ageratum corymbosum.* Phytochemistry. 1980; 19:2439. https://doi.org/10.1016/S0031-9422(00)91044-6.
12. Quijano L, Calderon JS, Gomez F, Escobar E, Rios T. Octa substituted flavones from *Ageratum houstonianum*. Phytochemistry.1985; 24:1085–1088. https://doi.org/10.1016/S0031-9422(00)83188-X.
13. Bowers WS, Ohta T, Cleere JS, Marsella PA. Discovery of Insect Anti-Juvenile Hormones in Plants: Plants yield a potential fourth-generation insecticide. Science.1976; 193: 542-547. https://doi.org/10.1126/science.986685.
14. Bowers WS, GerOffen. Pat. 2, 639-671. Chem Abstr. 1977; 87: 68153.
15. Staal GB. Anti Juvenile Hormone Agents. Ann Rev Entomol. 1986; 31: 391-429. https://doi.org/10.1146/annurev.en.31.010186.002135.
16. Pandey ND, Mathur KK, Pandey S, Tripathi RA. Effect of some plant extracts against pulse beetle. *Callosobruchus chinensis Linnaeus*. Ind J Entomol.1986; 48(1):85-90.
17. Do Rosário CJRM, Lima AS, Mendonça CJS, Soares IS, Júnior EBA, Gomes MN, Costa-Junior LM, Maia JGS, da Rocha CQ. Essential oil *Ageratum conyzoides* chemotypes and anti-tick activities. Vet Parasitol. 2023; 319:109942. https://doi.org/10.1016/j.vetpar.2023.109942.
18. Adams RP. Identification of essential oil components by gas chromatograph/mass spectrometry, 4th Etdn. Carol Stream, IL. Allured Publishing Corporation. 2007.
19. Brand-Williams W, Cuvelier ME, BersetC. Use of a Free Radical Method to Evaluate Antioxidant Activity LWT Food Science and Technology.1995;28:25-30. https://doi.org/10.1016/S0023-6438(95)80008-5.
20. Ilyasov IR, Beloborodov VL, Selivanova IA, Terekhov R P, ABTS/PP decolorization assay of antioxidant capacity reaction pathways. Int J Mol Sci. 2020; 21(3):1131.https://doi.org/10.3390/ijms21031131.
21. Mishra N, Rana K, Seelam SD, Kumar R, Pandey V, Salimath BP, Agsar D. Characterization and cytotoxicity of *Pseudomonas* mediated rhamnolipids against breast cancer MDA-MB-231 Cell Line. Front BioengBiotechnol.2021;9: 761266. https://doi.org/10.3389%2Ffbioe.2021.761266.
22. Chouhan S, Sharma K, Guleria S. Antimicrobial Activity of Some Essential Oils-Present Status and Future Perspectives. Medicines (Basel).2017;4(3):58. https://doi.org/10.3390/medicines4030058.
23. Lambert RJW, Skandamis PN, Coote P J, Nychas G J E. A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. J Appl Microbiol. 2001; 91(3):453-462. https://doi.org/10.1046/j.1365-2672.2001.01428.
24. Joshi RK. Chemical Composition of the essential oils of aerial parts and flowers of *Chromolaenaodorata* (L.) R. M. King and H. Rob. from Western Ghats region of North West Karnataka, India. J Essent Oil-Bear Plants. 2014;16:71-75. https://doi.org/10.1080/0972060X.2013.793971.
25. Robert V. Chemical composition of the essential oil of *Ageratum conyzoides* L. (Asteraceae) from Réunion. Flavour Fragr. J.1993; 8:257-260. https://doi.org/10.1002/ffj.2730080504.
26. Liu XC, Liu ZL. Evaluation of larvicidal activity of the essential oil of *Ageratum conyzoides* L. aerial parts and its major constituents against *Aedes albopictus*. J entomology Zoo Studies. 2014; 2(4): 345-350.
27. Ekundayo O, Laaksol, Hiltunen R. Essential Oil of *Ageratum conyzoides.* Planta Med.1988; 54(1): 55-57. https://doi.org/10.1055/s-2006-962336.
28. Riaz M, Khalid MR, Chaudhary FM. Essential oil composition of Pakistani *Ageratum conyzoides* L. J Essent Oil Res. 1995; 7:551-553. https://doi.org/10.1080/10412905.1995.9698584.
29. do Rosário CJRM, Lima ADS, Soares IS, Araújo RQ, Coimbra VCS, Andrade EHA, Sousa D M, Figueiredo P L B, Costa-Junior L M, da Rocha C Q. Seasonal and circadian evaluation of *Ageratum conyzoides* essential oil and its nematicidal activity against *Caenorhabditis elegans*. Acta Trop. 2024; 256:107274. https://doi.org/10.1016/j.actatropica.2024.107274.
30. Kouame BKFP, Toure D, Kablan L, Bedi G, Tea I, Robins R, Chalchat JC, Tonzibo F. Chemical constituents and antibacterial activity of essential oils from flowers and stems of *Ageratum conyzoides* from Ivory Coast. Rec Nat Prod. 2018; 12(2), 160-168. http://doi.org/10.25135/rnp.22.17.06.040.
31. Patil RP, Nimbalkar MS, Jadhav UU, Dawkar VV, Govindwar SP. Antiaflatoxigenic and antioxidant activity of an essential oil from *Ageratum conyzoides* L. J Sci Food Agric.2010; 90(4):608-614. https://doi.org/10.1002/jsfa.3857.
32. Nyemb N, Adele MDB, Njikam N, Hassane A. Antioxidant potential of aqueous leaf extract of *Ageratum conizoides* Linn. In diabetic rats, J Pharmacogn Phytother. 2009; 1(4):41-46.
33. Adebayo AH, Tan NH, Akindahunsi AA, Zeng G Z, Zhang Y M. Anticancer and antiradical scavenging activity of *Ageratum conyzoides* L. (Asteraceae). Pharmacogn Mag. 2010; 6(21):62-66. https://doi.org/10.4103/0973-1296.5996.
34. Sartori MRK, Atividadeantimicrobiana de frações de extratos e compost ospurosobtidos das flores de *Acmela brasiliensis* spreng (wedeliapaludosa) (Asteraceae) (Wedeliapaludosa) (Astraceae). 2005; 1-81.