



RESEARCH ARTICLE

Chemical composition and dual bioefficacy of *Chenopodium ambrosioides* essential oil: Anti-inflammatory and cytotoxic potential

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Abstract

Chenopodium ambrosioides L., a perennial aromatic herb of the family *Chenopodiaceae*, has long been recognized in ethnomedicine for its antimicrobial, anti-inflammatory and anticancer properties. This study aimed to analyze the chemical composition of its essential oil obtained from leaves collected in the Western Ghats, South India and to evaluate its cytotoxic and anti-inflammatory activities *in vitro*. Fresh leaves (2000 g) were hydro-distilled to yield 0.85 g (0.0425 %) of pale-yellow essential oil. The chemical profile was determined using Gas Chromatography-Mass Spectrometry (GC-MS), identifying 21 constituents representing 85.8 % of the total composition. α -Terpinene (38.2 %) and β -caryophyllene (11.7 %) were the predominant compounds, with notable amounts of *p*-cymene (9.7 %) and trans-carvyl acetate (5.8 %). Cytotoxicity was assessed against A549 human lung cancer cells using the MTT assay. The oil displayed dose-dependent inhibition of cell viability with an IC₅₀ value of 29 $\pm$ 1.5 μ g/mL. Anti-inflammatory potential was evaluated via the protein denaturation inhibition method using egg albumin, yielding an IC₅₀ value of 116.01 μ g/mL. These findings indicate that *C. ambrosioides* essential oil is rich in bioactive monoterpenes and sesquiterpenes, contributing to its significant cytotoxic and moderate anti-inflammatory effects. The results support its traditional medicinal use and suggest potential for developing natural therapeutic agents against cancer and inflammatory conditions. Further studies focusing on individual components and *in vivo* evaluations are warranted.

Keywords: α -terpinene; anticancer; anti-inflammatory; β -caryophyllene; *C. ambrosioides*; essential oil; Gas Chromatography–Mass Spectrometry

Introduction

Chenopodium ambrosioides (West Indian oosefoot, Mexican Tea, Epazote, American Wormwood) is a perennial herbaceous species of the family *Chenopodiaceae*. This aromatic plant is characterized by a distinctive camphoraceous smell, a red-shaded notched stem, lanceolate dentate leaves and elongated green inflorescences reaching up to 40 cm in length (1). Members of the family *Chenopodiaceae* are native of the East Mediterranean region and have a worldwide in Asia, China, India, Europe, North and South America, adapting to a wide range of environmental conditions. *C. ambrosioides* can serve as an antiviral aid due to its essential oils, which possesses bioactive properties with terpenes and flavonoids as key components (2, 3). Due to global warming and evolution, it has been proven that genes affect essential oil's effectiveness and its terpenes and these oils are effective towards viral with special attention to Cocksackie B4 virus which poses a threat as

a virus. The active compound in *cis*-ascaridole to have an inhibitory concentration of 21.75 μ g/mL with a high selectivity index of 74.34. Moreover, *C. ambrosioides* having high antibacterial and antifungal properties with minimum inhibitory concentrations. This oil poses great opportunity and further research around it poses great potential. Chemical analyses using the techniques of Gas Chromatography-Mass Spectrometry (GC-MS) and NMR (Nuclear Magnetic Resonance) showed that important components such as ascaridole, α -terpinene and *p*-cymene have potent antioxidant and cytotoxic activity (4-6). *C. ambrosioides* oil has been shown to possess versatility and application in pharmacological and agricultural contexts including the potential synergistic effects of the oil with conventional antimicrobials as well as use in the management of plant diseases (7, 8). While *C. ambrosioides* essential oil demonstrates promising therapeutic properties its toxicity toward normal human cells indicates that cautious

usage is necessary. A better understanding of *C. ambrosioides* essential oil's biochemical properties and safety profile will broaden its use across medicine and agriculture to food applications while uncovering new ways for its sustainable deployment (5). Although previous research has provided valuable insights, there is still insufficient investigation into the essential oil's anti-inflammatory action and cytotoxic effects when tested with A549 cells. This study aims to address this gap by evaluating both the anti-inflammatory properties of plant's oil and its cytotoxic effects on A549 cells *in vitro*.

Materials and Methods

Plant leaves collection

Leaves samples (2000 g) of *C. ambrosioides* were collected from Pollachi (10°39'32"N 77°00'29"E), South India. Verification and authentications were carried out by the Botanical Survey of India, Coimbatore, Tamil Nadu. The voucher specimen (HIT 011) is housed in the Chemistry Department.

Isolation of essential oil

Approximately 2000 g of *C. ambrosioides* leaves were hydrodistilled in a Clevenger-type apparatus for 4 hr. After drying the oil over anhydrous sodium sulfate, it was transferred to a container stored at 4 °C until GC-MS analysis. Four consecutive extractions of essential oil were performed to obtain the required amounts for further studies.

Gas Chromatography-Mass Spectroscopy analysis

The GC-MS analysis was conducted using a Thermo MS DSQ II system equipped with a standard non-polar column (30 m × 0.25 mm, film thickness 0.25 µm). Nitrogen was used as the carrier gas. The temperature program began at 70 °C with a retention time of 2 min, followed by an increase to 260 °C over a period of 10 min at a rate of 60 °C per min. The process concluded with a holding period at 350 °C, also lasting 10 min. Table 1 presents

CHN analysis results from an experimental spectrum. The Thermo Quadrupole mass spectrum system operated at a set ionizing energy of 70 eV, analyzing molecules within a mass range of 50-500 atomic mass units, while maintaining injector and transfer line temperatures at 280 °C (9).

Identification of chemical constituents

Compounds were identified by comparing their retention times and mass spectra with those in the National Institute of Standards and Technology mass spectral library v2.0g. The match percentage for each compound was recorded, with values above 90 % considered acceptable for identification. Additionally, retention indices (RI) were verified to fall within an acceptable tolerance of ±10 units. Quantification was performed using the external standard method, based on calibration curves derived from GC-MS analysis of the representative compounds (10, 11).

In vitro anticancer activity

The A549 lung cancer cell line was procured from the National Center for Cell Sciences in Pune, India. The cells were cultured using minimal essential medium (MEM), which ensured optimal growth conditions for the study. The anticancer efficacy was exclusively evaluated on A549 cells, as they represent a well-established human non-small cell lung cancer line. This cell line is extensively utilized in research due to its consistent growth characteristics, relevance to lung cancer pathology and suitability for drug screening assays.

IC₅₀ evaluation of cytotoxicity

For the MTT assay, 1×10^3 cells per well were seeded in a 96-well plate and grown for 48-50 hr until approximately 76 % confluence was reached. The original medium was removed and the cells were allowed to grow for an additional 1.5 days in a sample medium with varying concentrations of the test compound (10-50 µg/mL) prepared through serial dilution.

After treatment, each well was received 50 µL of dimethyl sulfoxide (DMSO) and the supernatant was decanted.

Table 1. Composition of essential oil on *C. ambrosioides*.

S. No.	List of compound	Retention time	Retention time estimated	Retention index reported	Composition (%)
1	α-terpinene	8.053	1015	1017	38.2
2	p-cymene	8.320	1021	1024	9.7
3	γ-terpinene	8.964	1061	1059	0.9
4	Trans- <i>p</i> -mentha-2,8-dien-1-ol	9.675	1125	1122	0.2
5	Trans-caranone	10.631	1198	1196	0.4
6	Thymol	12.341	1287	1290	0.3
7	Carvacrol	13.375	1295	1298	0.9
8	Trans-carvyl acetate	13.786	1341	1342	5.8
9	α-terpinyl acetate	14.097	1348	1349	1.5
10	Isobornyl propionate	14.508	1381	1384	0.5
11	β-caryophyllene	15.574	1421	1419	11.7
12	β-copaene	17.918	1433	1432	0.8
13	allo-aromadendrene	21.440	1462	1460	0.4
14	α-humulene	23.373	1474	1471	1.7
15	β-selinene	23.440	1488	1490	1.5
16	α-selinene	25.184	1501	1498	1.4
17	Elemicin	27.806	1559	1557	3.0
18	γ-asarone	28.084	1571	1574	2.6
19	Phytol	28.539	1946	1943	1.2
20	Geranyl benzoate	29.295	1960	1959	1.4
21	Eicosane	31.794	2002	2000	1.7
				Total	85.8

The plates were incubated for 10 min to solubilise the formazan crystals. Optical density measurement at 620 nm using a Thermo Multiskan EX ELISA multiwell plate reader (USA).

Evaluation of anti-inflammatory activity

Inhibition of egg albumin denaturation

The experimental procedure involved the preparation of 1 mL solutions of either essential oil (aqueous/ethanolic) or diclofenac sodium at concentrations ranging from 25 to 150 µg/mL. These solutions were combined with 1 mL of egg albumin (5 % in water) and incubated at 27 °C for 15 min. The control sample comprised egg albumin and distilled water. To evaluate the denaturation of egg albumin protein, the solution was subjected to heating at 70 °C for 10 min in a water bath. Following cooling to room temperature, the activity of the mixtures was assessed using spectrophotometry at a wavelength of 660 nm. Although diclofenac is referenced in the cytotoxicity assay, it is crucial to include a positive control, such as cisplatin, for comparative purposes to validate the bioefficacy of the tested compounds. Detailed comparative data should be presented to demonstrate the effectiveness of the essential oils relative to the positive control. The research employed adapted methods to evaluate the anti-inflammatory properties of essential oils. Comparative data should include the absorbance values of the essential oils and diclofenac against the positive control (cisplatin) to validate their bioefficacy and provide a clearer understanding of their potential therapeutic effects. The experiment performed three independent assessments for each test (12, 13).

Statistical analysis

The anti-inflammatory activity using the egg albumin denaturation assay was conducted in triplicate. Data were presented as mean ± standard deviation. The IC₅₀ value was determined using the sigmoidal dose-response curve fitting method with OriginPro 8.5 software.

Results and Discussion

Gas chromatography-Mass spectrometry analysis

The leaves of *C. ambrosioides* produced 0.85 g of pale-yellow essential oil from 2000 g of leaf material, resulting in an oil recovery rate of 0.0425 %. Through GC-MS analysis investigators examined oil components isolated from *C. ambrosioides* leaves according to Fig. 1. Twenty-one compounds were detected accounting for 85.8 % of the oil-yield (Table 1). The monoterpenoids consisted of monoterpene hydrocarbons (48.8 %), while sesquiterpenoids consisted of sesquiterpene hydrocarbons (17.5 %) and three monoterpene esters (7.8 %). The other compounds were phenylpropanoids (5.6 %), alkane (1.7 %), terpenoid ester (1.4 %), β-caryophyllene (11.7 %), *p*-cymene (9.7 %), trans-carvyl acetate (5.8 %) and elemicin (3.0 %). Other trace constituents were γ-asarone (2.6 %), eicosane (1.7 %), α-terpinyl acetate (1.5 %), β-selinene (1.5 %), geranyl benzoate (1.4 %), phytol (1.2 %), carvacrol (0.9 %), γ-terpinene (0.9 %) and isobornyl propionate (0.5 %).

Previous work has shown that the GC-MS analysis of the *C. ambrosioides* essential oil has produced a range of outcomes. A total of 44 compounds were isolated of which the major constituents include *cis*-ascaridole (58.32 %), *m*-cymene, α-terpinene and thymol. The highest intensity peak was observed for 4-carene with *o*-cymene as the second most intense peak at 41.46 %. In this present work, α-terpinene (38.2 %) was found to be the most dominant compound which was quite contrasting to the other available literature where *cis*-ascaridole and 4-carene were found to be the most abundant. In previous studies of *C. ambrosioides* oil was analyzed, they found that there were 24 compounds that constituted more than 98.5 % of the total oil (11). The detected compounds include; ascaridole, α-terpinene, *p*-cymene, neral, isoascaridole, geraniol and 2-carene (2.77 %). Similarly, essential oil was extracted chemically in *C. ambrosioides* from the Himalayan, which contained thirteen constituents making up 92.4 % of the oil (12). The main components have been identified and determined as, as caridol,

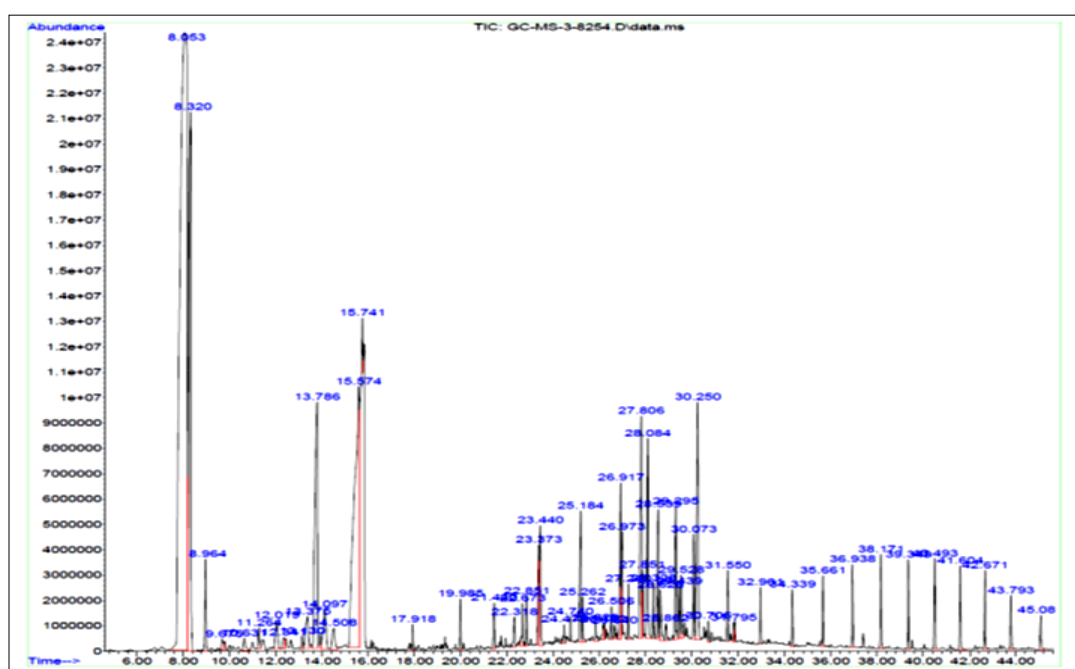


Fig. 1. Gas Chromatography-Mass Spectrometry chromatogram displays on essential oil composition from *C. ambrosioides* leaves.

isoascaridol, α -terpinene, p -cymene and γ -terpinene. The oil of this source was analyzed and the following 34 compounds were found which together constitute 95.78 % of the oil (13).

α -terpinene, isoascaridole and ascaridole were the main constituents in the analysis. The essential oil's principal compounds p -cymene and α -terpinene showed similar percentage distribution among samples from Himalayan and Kashmir Himalayan regions but their chemical components differed. The essential oil from *C. ambrosioides* showed good antibacterial and antifungal activity, with its main component α -terpinene highlighting the substance as an antiviral and antibacterial and antifungal agent (12).

In-vitro anticancer activity

In the current study we have assessed the *in-vitro* anticancer activity of *C. ambrosioides* essential oil on A549 cell line using MTT assay. Cancer cell viability was determined following an incubation period with variable essential oil concentration. Cell viability was concentration dependent reducing significantly in the lowest (10 $\mu\text{g/mL}$) and completely inhibiting cells in the highest concentration (50 $\mu\text{g/mL}$). Since the percentage cell inhibition as a function of the concentration was plotted, an IC_{50} value of $29 \pm 1.5 \mu\text{g/mL}$ was calculated (Fig. 2).

Essential oil from *C. ambrosioides*, shows good inhibited growth in liver cancer cell (SMMC-7721) by 94 % and 72 %, respectively in two concentration. *C. ambrosioides* oil had more anticancer activity than has been reported in earlier studies (13). *C. ambrosioides* essential oil demonstrates potential anticancer activity (A549 cell line) and has an IC_{50} $\mu\text{g/mL}$ and 125 $\mu\text{g/mL}$ (Table. 2) (14).

In the current research, the IC_{50} value of oil from *C. ambrosioides* from the southern region of the Western Ghats was significantly lower ($29 \pm 1.5 \mu\text{g/mL}$) than earlier reported, indicating that *C. ambrosioides* essential oil in southern region of the Western Ghats is more potent in case of *in vitro* anticancer activity. The findings indicate that it could be a promising candidate for cancer treatment at some point.

Table 2. Percent cell inhibition on essential oil of *C. ambrosioides*.

S. No.	Concentration ($\mu\text{g/mL}$)	Cytotoxicity %	
		Cisplatin	A549
1	0	98	97
2	10	60	75
3	20	35	61
4	30	24	48
5	40	21	36
6	50	19	23
IC_{50}		$29 \pm 1.5 (\mu\text{g/mL})$	

Evaluation of anti-inflammatory activity

Antibiotic activity of the protein ceases when its original structure changes during denaturation processes. Protein alteration serves as a critical component leading to inflammatory responses across multiple illnesses such as rheumatoid arthritis diabetes and cancer. Stopping protein denaturation emerges as an essential strategy to reduce inflammatory disease symptoms. The current research measured how the *C. ambrosioides* essential oil from leaves works against inflammation *in-vitro* by examining its effects on protein structures using the egg albumin denaturation protocol. The effect on egg albumin denaturation required analysis using various concentrations of essential oil combined with bovine serum albumin (egg albumin) at levels of 25, 50, 75, 100 and 150 $\mu\text{g/mL}$.

The researchers determined the IC_{50} value for protein inhibition to be 116.01 $\mu\text{g/mL}$ from the plotted concentration versus inhibition percentage curve displayed in Fig. 3. The essential oil demonstrated anti-inflammatory effects which showed concentration dependency because active flavonoids and triterpenoids were likely present. The reduced anti-inflammatory activity when utilizing *C. ambrosioides* with an IC_{50} level of 0.23 ppm (15). The current research presents a higher IC_{50} value for anti-inflammatory potential at 116.01 $\mu\text{g/mL}$.

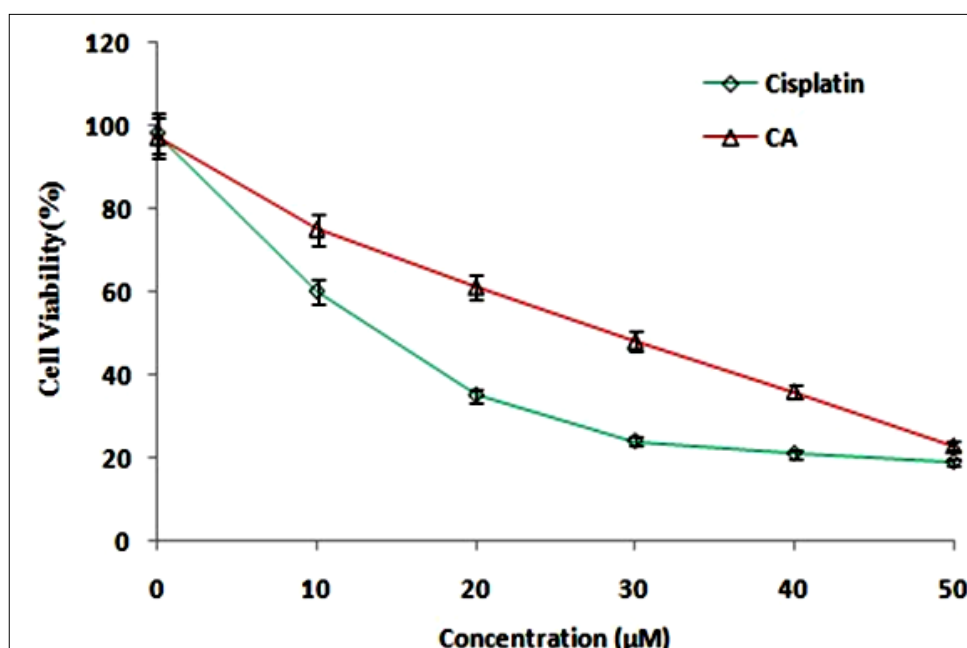


Fig. 2. Percent inhibition of essential oil of *C. ambrosioides* leaves of A549 cell line.

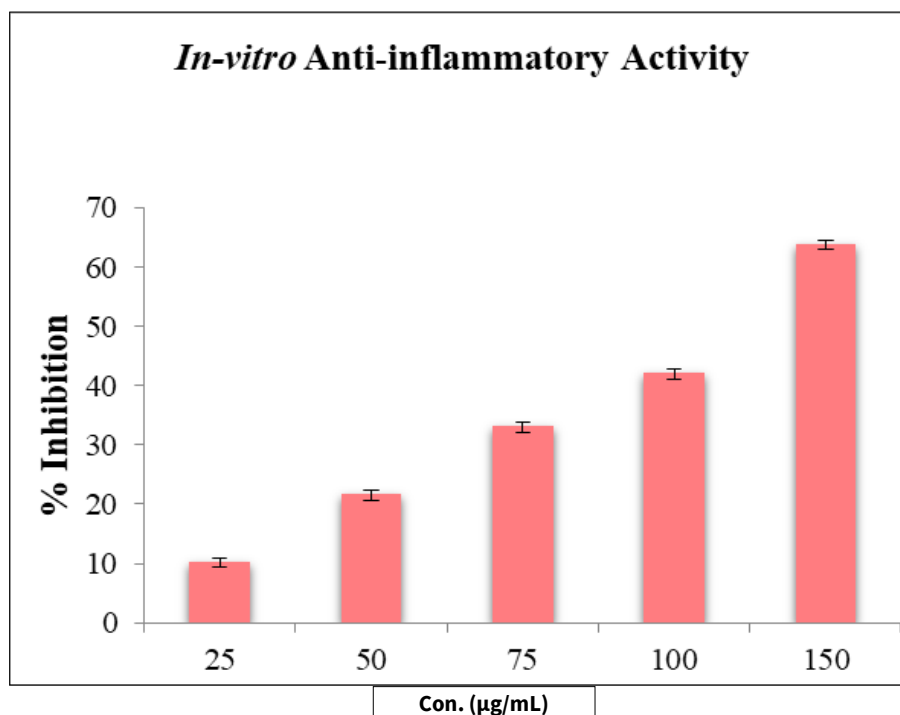


Fig. 3. *In vitro* anti-inflammatory activity essential oil on *C. ambrosioides* leaves.

Conclusion

The essential oil of *C. ambrosioides*, sourced from the Western Ghats, comprises 21 bioactive compounds, predominantly α -terpinene (38.2 %) and β -caryophyllene (11.7 %). This oil exhibits notable cytotoxicity against A549 lung cancer cells, with an IC_{50} value of 29 ± 1.5 μ g/mL and demonstrates substantial anti-inflammatory activity, with an IC_{50} of 116.01 μ g/mL, thereby affirming its therapeutic potential. Nevertheless, the *in vitro* nature of the study constrains the understanding of its effects *in vivo*. Additionally, the potential toxicity to normal cells necessitates cautious application and further safety assessments. Future research should incorporate *in vivo* trials to thoroughly evaluate both efficacy and safety. The development of formulations to enhance bioavailability and therapeutic efficacy is crucial. Furthermore, exploring synergistic effects with existing treatments and conducting tests on diverse cancer models will elucidate its broader applicability. Addressing these aspects will bolster the evidence supporting *C. ambrosioides* essential oil as a promising candidate for anticancer and anti-inflammatory therapies.

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Authors' contributions

VP and RP carried out the experiment, took observations, analysed the data and wrote the original draft. KV and BY participated in the design of the study and performed the statistical analysis. SD, MRK and SN contributed to review the manuscript, helped in editing, summarizing and revising the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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