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Research Article

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Evaluation of Anthelmintic Properties of *Kalanchoe petitiana* Essential Oil and Extracts on *Caenorhabditis elegans*

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Abstract

This study explores the chemical composition and anthelmintic activity of essential oils and extracts derived from the roots of *Kalanchoe petitiana* (Crassulaceae), a plant traditionally used in Ethiopian and Eritrean medicine. Essential oils were extracted through hydrodistillation and analyzed using gas chromatography-mass spectrometry (GC-MS), revealing 30 volatile compounds, with trans-myrtanol (43.23%), 1-octen-3-ol (5.43%), and bisabolene (6.02%) as the major components. The anthelmintic efficacy of the extracts and essential oil was evaluated in vitro against Caenorhabditis elegans nematodes. The methanol root extract exhibited significant nematocidal activity, achieving 91.5% mortality at a concentration of 10 mg/mL, while the chloroform root fraction demonstrated 61.7% efficacy at 2 mg/mL. The essential oil showed a potent effect with a 92.4% nematocidal rate at 60 µg/mL, closely comparable to the reference drug ivermectin (97.6% at 10 µg/mL). These results suggest that *K. petitiana* possesses substantial anthelmintic potential, likely due to its rich terpene content, particularly trans-myrtanol. This study provides the first report of trans-myrtanol identification from Kalanchoe species and supports using *K. petitiana* as a natural alternative for managing helminthic infections. Further research is recommended to isolate and characterize the active compounds and evaluate their vivo efficacy and safety.

Keywords: Anthelmintic activity; *Caenorhabditis elegans*; Essential oils; GC-MS; *Kalanchoe petitiana*; Trans-myrtanol.

1. Introduction

Helminth infections are among the most common neglected tropical diseases (NTDs) [1], affecting millions of people living in poverty [2]. Humans, animals, and crops are the main sources of helminth infections, constituting a health burden of approximately 2 billion people globally, more than a quarter of the world's population [3-6]. More than 550 million school-age children and about 270 million youngsters live in regions where these parasites are widely transmitted. Although infections are widespread, most cases are found in sub-Saharan Africa, the Americas, and Asia [7-9]. In Ethiopia, parasitic helminth infections are a significant public health issue. Numerous studies have revealed that the lower altitudes have a greater prevalence of parasitic illnesses [10-12]. These

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helminths are transmitted primarily due to poor hygiene practices, including exposure to fecal-contaminated water, soil, and food and wastewater reuse in agriculture [13-15]. These parasites can cause chronic debilitating disorders such as ankylostomoses, blindness, schistosomiasis, filarial worms, malnourishment, anemia, and retard growth in animals such as helminths of cattle [16, 17]. Helminthic parasite infections are among the most common *Ancylostoma duodenale*, *Ascaris lumbricoides*, *Necator americanus*, and *Trichuris trichiura* are the four principal human gastrointestinal nematodes, which are referred to as soil-transmitted helminthiasis (STH) [18-20]. Most anthelmintic drugs are limited to being active against trematodes, cestodes, and nematodes. The current treatment relies mainly on benzimidazole anthelmintics; levamisole, pyrantel, and praziquantel are the conventional anthelmintic drugs [21-23].

Studies have already reported reduced efficacy of human anthelminthic drugs [24]. Anthelmintic resistance is emerging, posing a significant risk to human and pastoral health resources at the current time and resisting the effects of medications [25]. Medicinal plants are a great source of effective treatment, and most current drugs are based on natural molecules [26, 27]. Many studies have been reported on the anthelminthic activity of plant extracts [28]. Screening of natural products holds great promise as drugs and can offer alternative treatment options to tackle anthelmintic resistance to conventional anthelmintic drugs [25, 29].

Kalanchoe is a genus belonging to the family Crassulaceae; it has traditional medicinal use in many parts of the world, particularly India, Africa, China, and Brazil [30, 31]. The juice of *Kalanchoe* plant species, or sometimes the raw plant material is used for the treatment of diseases like cheilitis, cracking lips in children, bruises, wounds, and boils in Brazil [32] for wound healing, syphilis, and expelling of tapeworm in Ethiopia [33] fever, abscesses, coughs, skin infections and cytotoxic activities [34, 35], cholera, urinary tract infections, whitlow in Africa and Asia [36], tissue damages, inflammatory conditions in Taiwan [37].

Kalanchoe petitiana, a species within the Kalanchoe genus, has been traditionally utilized in Ethiopian folk medicine, particularly for bone settings, where leaf juice is applied to fractures [38]. While specific studies on the chemical composition and pharmacological effects of *K. petitiana* are limited, research on related Kalanchoe species provides insights into potential bioactive compounds and therapeutic properties. Many Kalanchoe species contain bufadienolides, a class of C-24 steroids characterized by a six-membered lactone (α-pyrone) ring at C-17β. These compounds are known for their cardiotonic and antitumor activities [30]. Other Kalanchoe species have also been found to possess various bioactive compounds, including polysaccharides, flavonoids, sterols, ascorbic acid, organic acids, hydrocarbons, triterpenoids, and phenolic components. These constituents contribute to various pharmacological activities, such as anti-inflammatory, antimicrobial, and anticancer effects [32]. The genus *Kalanchoe* encompasses numerous species utilized in traditional medicine for various ailments. *Kalanchoe petitiana* has been employed as an anthelmintic, especially against tapeworms, and to treat conditions such as trachoma and syphilis. Previous studies have reported its antibacterial and antifungal properties, indicating a broad spectrum of bioactivity. However, the anthelmintic efficacy of *K. petitiana* against *H. contortus* remains underexplored. This study aims to fill this gap by evaluating the anthelmintic activity of *K. petitiana* essential oil and extracts and elucidating the potential mechanisms underlying their effects.

2. Materials and Methods

2.1. Plant material and botanical identification

Fresh leaves and roots of *Kalanchoe petitiana* were collected from Entoto Mountain near Addis Ababa, Ethiopia, in June 2021. Mr. Melaku Wondafrash, a senior botanist at the National Herbarium, College of Natural Sciences, Addis Ababa University, identified the plant where a voucher specimen (AD 002) was deposited.

2.2. Preparation of crude extracts and fractionations

The plant's fresh leaves and roots were dried under shade and ground in a mortar. After the drying, 160 grams of powdered root material was macerated with 80% methanol to obtain the hydroalcoholic crude extract in three rounds. The leave was firstly defatted with 99% hexane and again macerated with 80% methanol twice. The methanol root crude extract was further fractionated using hexane, chloroform, methanol, and water solvents. The fractions were concentrated under a vacuum in a rotary evaporator.

2.3. Essential Oil Extraction

The essential oil from the root of *Kalanchoe petitiana* was extracted using hydrodistillation under optimal conditions. A total of 150 grams of root material was combined with 500 mL of distilled water in a 2-liter flask. The setup was placed on a balloon heater and connected to a condenser to facilitate the collection of essential oils over a 4-hour distillation period. Upon completion, two distinct phases were observed in the Clevenger apparatus: a lower aqueous phase and an upper organic phase containing the essential oil, less dense than water. The essential oil was then separated, collected, and stored in sealed vials at 4°C.

2.4. Gas Chromatography-Mass Spectrometry (GC-MS)

The GC-MS analysis was performed using gas chromatography (GC-7890B manufactured by Agilent Technologies) coupled with mass spectrometry (MS-5977A model manufactured by Agilent Technologies) under the following operating conditions: DB-1 fused silica capillary column 25 m x 0.23 mm x 0.25 µm film thickness; carrier gas: He; flow rate: 0.8 ml/min; oven temperature: 50-200°C with a rate of 5°C per minute.

2.5. Preparation of test substances

The test substances were prepared in a 4% DMSO and M9 buffer solution. The test samples were evaluated at two different concentrations. Specifically, the crude fractions were tested at 2 mg/mL and 10 mg/mL, the chloroform fractions at 1 mg/mL and 2 mg/mL, and the essential oil at 15 µg/mL and 60 µg/mL.

2.6. Caenorhabditis elegans nematode and culture methods

The nematode *Caenorhabditis elegans* was maintained under standard laboratory culture conditions at a controlled temperature of 20 °C. The N2 strain of *C. elegans* was sourced from the *C. elegans* Genetics Center, housed at the Leibniz Institute of Plant Biochemistry, Germany. The worms were routinely cultured using established protocols to ensure optimal growth and viability for experimental procedures.

2.7. Anthelmintic assay

The anthelmintic assay was conducted on the model nematode Caenorhabditis elegans following the method described by Thomson et al. (2012). The nematodes were cultured on Nematode Growth Medium (NGM) petri plates, using the uracil auxotrophic Escherichia coli strain OP50 as a food source. After four days of cultivation, they were harvested by rinsing each plate twice with 2 mL of M9 buffer and transferring the suspension to a 15 mL Falcon tube. The worm suspension was centrifuged at 800 × g for 1 minute, after which the supernatant was removed. The nematodes were rewashed with 2 mL of M9 buffer under the same conditions and resuspended in 2 to 8 mL of M9 buffer, depending on the number of worms. A 10 µL aliquot of penicillin-streptomycin solution (10 mg/mL) was added to the suspension.

The assay was performed in 384-well plates, with the nematode concentration adjusted to 20–30 worms per 20 µL. The outer wells were filled with 40 µL of water to minimize evaporation. Each well received 20 µL of worm suspension with 20 µL of the test solution. After a 30-minute incubation, each well's number of live and dead nematodes was counted using an inverted cell culture microscope (Olympus CKX41, Olympus Life Science, Waltham, Massachusetts, USA). DMSO (2%) served as the negative control, while ivermectin (10 µg/mL) was used as the positive control. All assays were performed in triplicate.

3. Results and Discussion

3.1. Extraction Process and Chemical Composition

The hydroalcoholic extraction of the root and leaves of *Kalanchoe petitiana* yielded 9% and 16% w/w, respectively. The root extract was further fractionated into hexane, chloroform, methanol, and aqueous fractions, with the chloroform fraction yielding the highest percentage at 30%. The essential oil, extracted from the root by hydrodistillation, was obtained at a yield of 0.1%. GC-MS analysis identified 30 volatile compounds, comprising 96.45% of the oil. Major components included trans-myrtanol (43.23%), 1-octen-3-ol (5.43%), myrtenol (4.2%), nonenal (5.9%), bisabolene (6.02%), and viridiflorol (1.53%). The percentage yields of the crude extracts and fractions are presented in **Table 1**. The chemical compositions of the essential oil isolated from the roots of *K. petitiana* is summarized in **Table 2**. The GC-MS spectrum, the essential oil is shown in **Figure 1**.

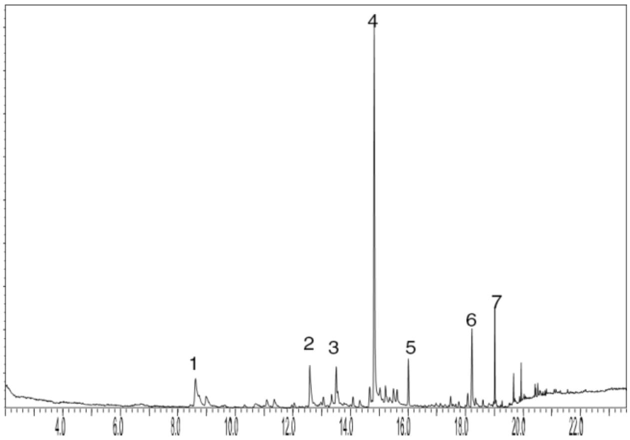

Figure 1: Gas chromatography-mass spectroscopy (GC-MS) of essential oil of *Kalanchoe petitiana* (Crassulaceae) root: 1-Octen-3-ol (**1**), Nonen-1-al (**2**), Myrtenol (**3**), Trans-myrtanol (**4**), Cyclohexasiloxane, Dodecamethyl (**5**), E-Bisabolene (**6**), Hexasiloxane (**7**).

Table 1: Percentage yields (weight by weight) of the hydroalcoholic extract and solvent fractions of *Kalanchoe petitiana*.

Crude extracts	Yield % (w/w)	Fractions	Yield % (w/w)
Methanol root extract	9	Hexane root fraction	20
Methanol leave extract	16	Chloroform root fraction	30
		Methanol root extract	35
		Aqueous root fraction	15

Table 2: Chemical compositions of the essential oil isolated from the roots of *K. petitiana* collected from Mount Entoto, Addis Ababa

No.	Components[a]	KI[b]	KI-lit[c]	%	Method of identification
1	1-Octen-3-ol	972	969	5.40	KI-lit & MS
2	Furan	977	984	2.50	KI-lit & MS
3	3-Octanol	987	979	3.00	KI-lit & MS
4	Trans-2-Octenal	1,002	1,052	0.30	KI-lit & MS
5	2-Nonanone	1,074	1,087	1.40	KI-lit & MS
6	Nonanal	1,085	1,100	1.70	KI-lit & MS
7	3-Pinanone	1,119	1,109	0.70	KI-lit & MS
8	Cis-Nonen-1-al	1,144	1,144	5.90	KI-lit & MS
9	1-Nonanol	1,161	1,159	0.90	KI-lit & MS
10	Cis-Myrtanol	1,165	1,188	1.30	KI-lit & MS
11	Cis-Ocimene	1,178	1,164	2.20	KI-lit & MS
12	Myrtenol	1,185	1,194	4.20	KI-lit & MS
13	n-Decanal	1,187	1,201	1.70	KI-lit & MS
14	Cis-Sabinene	1,212	1,219	1.10	KI-lit & MS
15	Benzaldehyde	1,223	1,230	1.20	KI-lit & MS
16	Cis-Myrtanol	1,240	1,250	2.10	KI-lit & MS
17	Trans-Myrtanol	1,247	1,258	41.10	KI-lit & MS
18	P-Menth-1-en-7-al	1,257	1,273	0.70	KI-lit & MS
19	2-Undecanone	1,273	1,285	0.80	KI-lit & MS
20	(4-propan-2-ylphenyl)methanol	1,279	1,289	1.60	KI-lit & MS
21	Cyclohexasiloxane	1,305		3.10	MS
22	1,1,6-trimethyl-2H-naphthalene	1,359	1,355	0.50	KI-lit & MS
23	Lavandulyl Isobutanoate	1,426		0.50	MS
24	2-(4-Isobutylphenyl)-1-Propanol	1,532	1,532	1.30	KI-lit & MS
25	Trans-Bisabolene	1,537	1,528	6.00	KI-lit & MS
26	Neryl Acetone	1,542		1.30	MS
27	Copaen-4-alpha-ol	1,578		0.10	MS
28	Hexasiloxane			0.40	MS
29	Caryophyllene oxide	1,588		0.30	MS
30	Viridiflorol	1,593	969	1.50	KI-MS
	Total			94.80	

Notes: [a] Compounds listed in order of elution; [b] KI is the Kovats retention indices determined relative to a series of n-alkanes (C9–C29) on a non-polar (HP5-MS capillary) column; [c] KI-lit is the Kovats retention indices with those reported in the literature on the same column under conditions listed in the Experimental section; compounds are identified by comparing their Kovats retention indices and mass spectra (MS) with those listed in the Adams, NIST, Wiley mass spectral libraries and laboratory's database.

3.2. Anthelmintic Activity

The *in vitro* anthelmintic activities of the hydroalcoholic crude extracts and essential oil from *K. petitiana* were assessed using the nematode *Caenorhabditis elegans*. The methanol root extract exhibited the highest nematocidal activity at 10 mg/ml, achieving 91.5% mortality. The chloroform fraction of the root showed significant activity at 2 mg/ml (61.7% mortality). The essential oil demonstrated a potent effect at 60 µg/mL, with a 92.4% nematocidal rate, comparable to the reference drug ivermectin, which showed 97.6% efficacy at 10 µg/mL with the results presented in **Table 3**. The results showed that the extract-treated *Caenorhabditis elegans* exhibited dose-dependent mortality, whereas those treated with ivermectin as the reference drug showed similar effects [39].

This is the first study to explore the anthelmintic activity and GC-MS analysis of the volatile oil extracted from *K. petitiana*. The crude extracts of this plant were meticulously prepared utilizing 80% methanol as the hydroalcoholic solvent, with defatting of the plant leaves occurring prior to the hydroalcoholic maceration process. The essential oil was meticulously extracted through hydrodistillation using the Clevenger apparatus.

The highest anthelmintic efficacy was observed in the methanol crude extract and chloroform fraction of the root of *K. Petitiana*, with effectiveness rates of 91.5% at 10 mg/ml and 61.7% at 2 mg/ml, respectively (**Table 3**). Notably, the essential oil extracted from the root of *K. Petitiana* demonstrated remarkable anthelmintic potency at a concentration of 60 µg/mL, achieving an efficacy of 92.4%, a result comparable to the reference drug, ivermectin, which showed 97.6% efficacy. To our knowledge, there have been no previous reports on the anthelmintic properties *of K. Petitiana* extracts and essential oil. In the realm of research focusing on the anthelmintic potential of *Kalanchoe* species, it is noteworthy that *Kalanchoe pinnata* exhibited a significant reduction in *C. elegans* motility in a study conducted in Nepal. [40]. The composition of the essential oil, which predominantly consists of terpenes and terpene alcohols, is of particular interest. Intriguingly, the analysis unveiled that trans myrtanol comprised 43.23% of the dominant component, marking the first instance of myrtanol isolation from Kalanchoe species. Furthermore, the essential oil extracted from the leaves of *Kalanchoe petitiana* through hydrodistillation is primarily characterized by diterpene alcohols, constituting 35.6% of its composition.[41].

Table 3: Effect of crude extracts, solvent fractions, and essential oil of *K. petitiana* on survival of adult *C. elegans* after 30 minutes of exposure

Samples	Types of extracts	Doses	% of death of C. elegans nematode (death% ± SD)
Crude extracts	Root	2 mg/ml	44.2% ± 1.6
		10 mg/ml	91.5% ± 2.16
	Leave	2 mg/ml	NA
		10 mg/ml	17.7% ± 0.1
Methanol root fractions	Hexane FR	1 mg/ml	NA
		2 mg/ml	NA
	Chloroform FR	1 mg/ml	34.5% ± 1.6
		2 mg/ml	61.7%±1.9
	Methanol FR	1 mg/ml	NA
		2 mg/ml	22.1% ± 0.4
	Aqueous FR	1 mg/ml	NA
		2 mg/ml	NA
Essential oil	Essential oil	15 µg/mL	45.3% ± 0.8
		60 µg/mL	92.4% ± 1.6
Ivermectin	Reference	10 µg/mL	97.6% ± 2.6
DMSO	Control group	2%	2.2% ± 0.1

FR = fraction; NA = no activity; SD: Standard deviation (n = 3); ($P < 0.05$).

The anthelmintic activity observed in *K. petitiana* extracts and essential oil is likely attributed to bioactive compounds, particularly terpenes and terpene alcohols, which are well-known for their biological effects [42]. The high concentration of trans-myrtanol in the essential oil, comprising 43.23% of the total composition, may significantly contribute to the anthelmintic effects observed [43]. Myrtanol, isolated from *Thymus tosevii* oil, was evaluated against harmful intestinal parasites and inhibited the growth of the organisms [44]. This study marks the first report of trans-myrtanol isolation from *Kalanchoe* species. The findings align with previous studies on other Kalanchoe species, such as K. pinnata, which also demonstrated significant anthelmintic activity against *C. elegans* [45]. These results suggest that *K. petitiana* could serve as a potential source for developing novel anthelmintic agents, especially in light of increasing resistance to conventional drugs like benzimidazoles. The results of this study highlight the promising anthelmintic properties of *K. petitiana*, particularly through its root extracts and essential oil. The high efficacy observed in the methanol root extract and the essential oil is particularly notable, given the increasing global challenge of anthelmintic resistance. The anthelmintic efficacy of *K. petitiana* observed in this study is comparable to that of widely used conventional anthelmintics like ivermectin [46]. Specifically, the essential oil of K. petitiana achieved a 92.4% nematocidal rate at a concentration of 60 µg/mL, closely matching ivermectin's efficacy of 97.6% at 10 µg/mL. This indicates that the bioactive compounds in K. petitiana, particularly trans-myrtanol, could offer a natural alternative to synthetic anthelmintics.

The mechanism by which these terpenes exert their effects may involve disrupting the parasite's cell membranes or interfering with key enzymatic pathways [47]. However, further studies are needed to confirm these mechanisms.

Helminthic infections continue to pose a major public health challenge, especially in low-income regions where access to conventional anthelmintic treatments is limited [48]. Therefore, the discovery of potent anthelmintic activity in K. petitiana is highly relevant, offering a potential source of affordable and effective treatment [49]. Given that *Kalanchoe* species are traditionally used in various parts of the world for treating parasitic infections, this study provides scientific validation for these ethnomedicinal uses. It supports the integration of *K. petitiana* into modern herbal medicine practices [50].

Moreover, the use of plant-based anthelmintics could reduce the reliance on synthetic drugs, thereby mitigating the risk of further development of resistance [51]. Applying *K. petitiana* as a natural deworming agent could be particularly beneficial in agriculture, where parasitic infections in livestock are a major concern. By reducing animal parasite loads, *K. petitiana* extracts could improve livestock health and productivity, which is crucial for food security in many developing countries [52, 53]. The results of this study are consistent with findings from studies on other *Kalanchoe* species. For instance, *Kalanchoe pinnata* has also been reported to exhibit significant anthelmintic activity, supporting the potential of this genus as a source of natural antiparasitic agents [45]. However, the high content of trans-myrtanol in *K. petitiana* is particularly noteworthy, as this is the first report of its isolation in a *Kalanchoe* species. This suggests that different *Kalanchoe* species may have unique phytochemical profiles, which could be exploited to develop species-specific therapeutic agents.

3.3. Future Research Directions

While the study provides a strong foundation for using *K. petitiana* in anthelmintic therapy, several areas warrant further investigation. First, advanced chromatographic techniques such as HPLC should be employed to isolate and identify the specific active compounds responsible for the observed anthelmintic activity. Understanding these compounds' exact composition and concentration will be critical for developing standardized herbal formulations.

Furthermore, *in vivo* studies are essential to assess the safety and effectiveness of *K. petitiana* extracts in animal models. These investigations would offer important insights into pharmacokinetics, optimal dosage, and possible adverse effects of these natural compounds. Finally, exploring the synergistic effects of combining *K. petitiana* extracts with other known anthelmintics could enhance their efficacy and reduce the likelihood of resistance development.

4. Conclusion

The findings of this study suggest that *Kalanchoe petitiana*, particularly its root extracts and essential oil, holds significant promise as a natural anthelmintic agent. The high content of bioactive terpenes, especially trans-myrtanol, contributes to its potent antiparasitic effects. With further research and development, *K. petitiana* could be integrated into therapeutic protocols for treating helminthic infections, offering an affordable and effective alternative to conventional drugs.

Abbreviations

HPLC High-Performance Liquid Chromatography
GC/MS Gas Chromatography/ Mass spectrometer
DMSO Dimethylsulfoxide

Ethics approval and consent to participate

Not applicable.

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Consent for publication

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Competing interests

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Using artificial intelligence chatbots

There was no use of artificial intelligence in the making of this article.

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