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Chemical Composition and Bioactivities of *Ferulago setifolia* K. Koch. Essential Oil: Antifungal, Insecticidal and Antiproliferative Potential

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ABSTRACT

The essential oil extracted from the aerial parts of *Ferulago setifolia* K. Koch was analysed using GC/MS, revealing 33 compounds, with 2,3,4-trimethyl benzaldehyde (41.24%), α-pinene (21.58%) and sabinene (10.46%) as major constituents. The biological activity profile of essential oils, including antifungal, insecticidal and antiproliferative activities, was documented for the first time. The oil demonstrated significant antifungal activity, completely inhibiting the growth of *Verticillium dahliae* at higher doses and showing dose-dependent inhibition against *Alternaria solani*. It also exhibited moderate insecticidal activity against *Rhyzopertha dominica* and *Tribolium confusum*, achieving a mortality rate of up to 49.3% after 48 h at a 5% concentration. Furthermore, the essential oil showed potent antiproliferative effects against a wide range of cancer cell lines, including lung (Calu1), breast (MCF7), colon (HT29) and gynaecological (HeLa) cancers, while maintaining low cytotoxicity on normal cell lines (MRC5, FL). The IC_{50} values ranged from 8.13 μg/mL (A2780) to 92.88 μg/mL (HT29), with notable results such as 22.84 μg/mL for Calu1 and 58.26 μg/mL for MCF7. Compared to the standard anticancer drug 5-fluorouracil, the essential oil exhibited superior or comparable activity against certain cancer cell lines. The results showed that essential oils have specificity against cancer cell lines. These findings suggest that the essential oil of *F. setifolia* holds potential as a natural source for antifungal, insecticidal and antiproliferative agents. The results contribute significantly to the pharmacological and agricultural potential of this species.

1 | Introduction

Plant-derived essential oils have gained considerable attention as a sustainable and eco-friendly alternative in pharmacology, agriculture and the food industry. The natural antimicrobial, insecticidal and anticancer properties of essential oils make them highly valuable in addressing issues associated with synthetic chemicals, such as toxicity, environmental harm and the development of resistance. The diverse chemical compositions of essential oils allow for multifunctional applications, making them particularly promising for human health.

The genus *Ferulago* W. D. J. Koch (Apiaceae) is represented by 48 species spread across the Balkans, Eastern Europe, the Transcaucasus, the Middle East, North Africa and the Mediterranean Basin [1]. A total of 34 species from the *Ferulago*

genus have been recorded in Turkey with a 50% endemism ratio [2]. In Türkiye, species of the genus known as 'kişniş' or 'çakşır' are traditionally valued for their medicinal, culinary and aromatic properties [3]. Due to their sedative, tonic and digestive properties, they are often used as decoctions and infusions in alternative medicine [4]. Additionally, they serve as spices and flavouring agents in soups [5]. Phytochemical studies have revealed that the biological activities of these *Ferulago* species, including anti-inflammatory, antioxidant, antitumoral, antimicrobial, antidiabetic, aphrodisiac and antiproliferative properties [6–11], are attributed to metabolites such as coumarins [12, 13], flavonoids [14], organic acids [15] and particularly terpenes, which are abundant in the essential oil fraction [16].

F. setifolia is a perennial plant that grows to a height of 40–100 cm and is native to Turkiye and Transcaucasia. It is completely smooth and hairless. The leaves are finely divided, featuring 3–4 levels of branching, giving them a narrow, lance-shaped appearance. The flowers are arranged in a terminal, branched cluster. The bracts are narrow, lance-shaped, and have a yellowish colour. The fruits are elliptical, measuring about 13 mm long and 8 mm wide, with a base that appears flat or slightly indented (Figure 1). *F. setifolia* can be distinguished from *F. stellata*, which closely resembles it, by its more rayed central umbels and significantly longer and narrower bracts and bracteoles [17].

F. setifolia is used as a sedative, digestive, carminative and food additive for flavouring soup and meatballs by local peoples and is known as 'kıl kişniş' Although the biological activity profile of *F. setifolia* extract, including antioxidant, antibacterial and antiproliferative activity [3], has been reported previously, the biological activity potential of its essential oil is not well documented. While some studies have investigated the chemical composition of essential oil [16, 18, 19], its pharmacological and agricultural applications are yet to be fully elucidated. This study contributes to the biological activity perspective of the *F. setifolia* essential oil. The objectives of this research are threefold: (i) to analyse the chemical composition of the essential oil extracted from *F. setifolia*; (ii) to evaluate its antifungal and insecticidal properties for agricultural applications; and (iii) to assess its antiproliferative potential against various cancer cell lines for pharmacological applications.

FIGURE 1 | The appearance of *Ferulago setifolia* plant in its natural habitat.

2 | Materials and Methods

2.1 | Plant Materials

Plant materials were collected from Ergan Mountain, Erzincan/Turkey, in June 2021 (39°38′46.16″ N, 39°30′35.55″ E). The voucher specimens were authenticated by Prof. Dr. Ali Kandemir and deposited at the Herbarium of the Department of Science and Art Faculty, Erzincan Binali Yıldırım University, Erzincan, Türkiye (EBYU No: 000031).

2.2 | Essential Oil Extraction

Essential oil extraction was performed using a Neo-Clevenger-type apparatus. Fresh aerial parts of *F. setifolia* (100 g, finely chopped) were transferred into a 1 L round-bottom flask, and 500 mL of distilled water was added to ensure complete submersion of the plant material. The apparatus was operated under reflux for 3 h, allowing for continuous steam distillation. The essential oil was then separated from the aqueous phase using a liquid–liquid partition within the Neo-Clevenger system. The collected essential oil was dried over anhydrous sodium sulfate (Na_2SO_4) to remove any residual moisture, followed by filtration. Finally, the essential oil was transferred into dark glass vials and stored at +4°C until further analysis to prevent oxidation and degradation.

2.3 | Analysis of the Oil Samples

The essential oil was obtained in a pale yellow colour, with a yield of 4.2% based on fresh weight. GC/MS analyses were performed using a Thermo Scientific Trace 1310 GC/MS system equipped with an HP-5MS capillary column (30 m × 0.25 mm, 0.25 mm ID and 0.25 μm film thickness). Helium was used as a carrier gas in split mode by 50:1 at a 1.2 mL/min flow rate. The column oven was heated from an initial temperature of 60°C (held for 3 min) to 200°C at 3°C/min (no hold), then to 240°C at 5°C/min (held for 5 min). All heated zones, including the ion source, transfer line and injection port, were set to 280°C. The mass spectra were recorded at 70 eV ionisation energy in EI mode with a 3-min solvent delay. An essential oil solution (5%, in acetone) was injected in a 1 μL volume into the preheated injection site. The retention indexes (RI) were calculated for all components using the Van den Dool and Kratz equation based on homologue *n*-alkane series retention times. The compounds

were identified using Wiley and NIST2004 MS libraries. The relative peak area percentages of each compound were calculated based on the MS chromatograms [20].

2.4 | Antiproliferative Activity

The antiproliferative activity of crude essential oil was evaluated against lung cancer (A549 and Calu1), breast cancer (MCF7), colon cancer (HT29) and bone cancers (SW1353, MG63, Saos2), in addition to gynaecological cancers (A2780, A2780ADR and HeLa) and normal cell lines (MRC5 and FL). All cell preparations were carried out under sterile conditions in a laminar flow cabinet. MCF7, A2780, A2780ADR and other gynaecological cancer cell lines were cultured in RPMI1640 medium supplemented with 10% FBS and 2% PenStrep at 37°C in a controlled 5% CO_2 environment, while DMEM was used for the remaining cell lines. Initially, cells were seeded at a density of 10,000 cells per well, followed by a 16-h pre-incubation period before treatment with the test materials. After a 24-h exposure, cell proliferation was measured using the MTT assay, according to previous reports [3, 21]. Test compounds were prepared in 5% DMSO at concentrations ranging from 1.96 to 125 μg/mL. The IC_{50} values were determined via logarithmic analysis of absorbance readings at 570 nm.

2.5 | Cytotoxicity Test

The LDH cytotoxicity assay was performed using a commercially available LDH cell cytotoxicity kit, following the manufacturer's protocol. Briefly, the enzymatic activity of LDH was assessed by measuring the formation of formazan. The percentage of cytotoxicity was calculated using the following formula: % Cytotoxicity = [(Substance Absorbance − Low Control/High Control − Low Control) × 100].

2.6 | In Vitro Antifungal Activity

The antifungal potential of the essential oil derived from *Ferulago setifolia* was tested against two fungal pathogens: *Verticillium dahliae* and *Alternaria solani*. Fungal cultures, aged 7 days and grown on potato dextrose agar (PDA) at 25°C ± 2°C, were used. PDA medium was sterilised via autoclaving, cooled to 40°C, and poured into 60 mm Petri dishes. Mycelial plugs taken from the fungal cultures were spiked on the PDA plates. Sterile filter papers (5 mm) affixed to the dish lids were treated with varying concentrations of the essential oil using a micropipette: 0 (control), 0.6, 1.2, 2.4, 4.8 and 9.6 μL per dish. The dishes were then sealed to prevent oil evaporation and incubated at 25°C ± 2°C for 7 days. Fungal growth was assessed by measuring the radial expansion of the colonies. Growth inhibition was calculated using the formula: $\text{Inhibition}\,\% = \frac{C-T}{C} \times 100$, where C represents the average radial growth of the control group, and T represents the average radial growth of the treated samples. Each concentration was tested in triplicate, and the entire experiment was repeated twice for consistency. This methodology provided a robust assessment of the antifungal efficacy of *F. setifolia* essential oil against these pathogens.

2.7 | Insecticidal Activity

The insecticidal activity of essential oil of *F. setifolia* was evaluated using a contact toxicity experiment against *Rhyzopertha dominica* (Fabricius) (Coleoptera: Bostrichidae) and *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae). The insects were cultivated using a nutrient mixture made of crushed wheat and dry yeast (9:1). Adults aged 7–14 days were selected for experiments, with their emergence monitored daily to ensure uniformity, approximately 1 week after the eggs were introduced into the food jars for testing. A micro applicator was used to apply 1 μL of the essential oil solution (1%, 2.5% and 5% in acetone) to the dorsal thorax of each insect. Control groups received the same volume of acetone. The treated insects were placed into 25 mL glass tubes containing approximately 10 g of the prepared wheat–yeast mixture and incubated at a temperature of 27°C ± 2°C. Mortality was recorded after 24 and 48 h of exposure. The results were expressed as percent mortality ± SD of three independent experiments.

2.8 | Statistical Analysis

The significance of the differences between treatments in the trials was determined by analysis of variance (ANOVA), and the averages were compared using the DUNCAN test and Analysis of variance was performed with the obtained data, and in addition, differences between treatments were analysed with the Tukey multiple comparison test. All statistical analyses were carried out with the help of the MINITAB Release 18 package program. Statistical analyses were performed using the SPSS 15 computer program.

3 | Results and Discussion

3.1 | Essential Oil Analysis

The essential oil obtained is pale yellow in colour, with a yield of 4.2% based on fresh weight. The yield of *F. setifolia* essential oil (4.2%) is within the range reported for other *Ferulago* species but exhibits some variations depending on species and environmental factors. For instance, *Ferulago macrosciadea* essential oil has been reported to yield between 2.5% and 4.8% [19], whereas *F. angulata* yields 3.1% to 5.3% under different conditions [22].

The essential oil composition of *F. setifolia* was analyzed, revealing a diverse range of volatile compounds (Figure 2). The results are presented in Table 1. Totally, 33 components representing 98.20% of the essential oil content were identified, with monoterpenes and oxygenated monoterpenes being the predominant groups, constituting 46.51% and 50.27% of the total content, respectively. 2,3,4-trimethylbenzaldehyde was found to be the major component at 41.24% of the total oil, followed by α-pinene, accounting for 21.58%. Sabinene (10.46%), 2,3,6-trimethylbenzaldehyde (5.20%), and α-myrcene (4.05%) also represented significant portions of the monoterpenes, contributing characteristic odor and bioactivity potentials. Oxygenated monoterpenes were found in smaller amounts, with

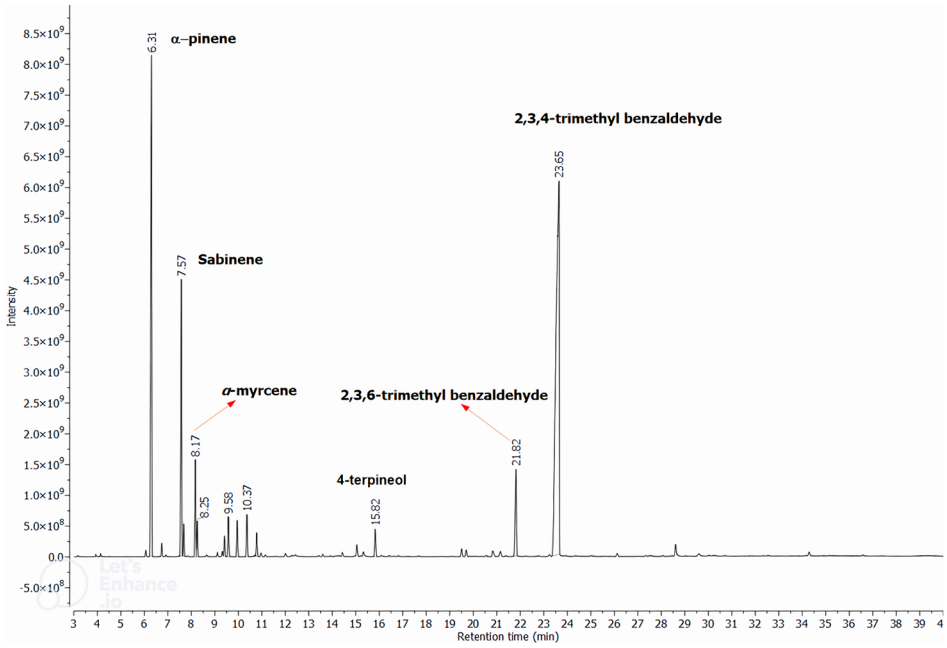

FIGURE 2 | GC/MS chromatogram of essential oils of *Ferulago setifolia*.

compounds such as cis-ocimene (1.88%), 4-terpineol (1.41%), and β-ocimene (1.59%). Sesquiterpenes were also found in lower amounts, such as germacrene-D (0.70%) and spathulenol (0.23%). Although present in smaller quantities, these components may still significantly influence the biological activity profile of crude essential oil due to their known bioactive properties.

In the present study, it is reported that the 2,3,4-trimethyl benzaldehyde is the most abundant component, at 41.24% of the oil, whereas Polat et al. reported a higher concentration of 2,4,5-trimethyl benzaldehyde at 77.8% and a lower concentration of 2,3,4-trimethyl benzaldehyde at 6.20%, with notable differences in the relative abundances of these compounds [18]. Additionally, our findings indicate a higher percentage of α-pinene (21.58%) compared to their study. The differences can be explained by variations in temporal and geographic conditions. Additionally, the time of year, growth stage, and the parts of the plant that are harvested can significantly impact the composition of essential oils. Several studies have reported that the essential oils of some *Ferulago* species contain benzaldehyde derivatives, including 2,3,4-trimethyl benzaldehyde, 2,4,5-trimethyl benzaldehyde, and 2,4,6-trimethyl benzaldehyde as major components. These aldehydes were found in different plant parts of eight *Ferulago* species, varying from 5.25% to 92.7% [5, 23–26], supporting the current study.

In conclusion, the present study highlights the chemical diversity and majority of specific benzaldehyde derivatives, particularly 2,3,4-trimethylbenzaldehyde, in the essential oil of *F. setifolia*. The findings are fully comparable with previous reports on the chemical profiles of various *Ferulago* species, while also demonstrating notable differences in relative abundances. The high content of monoterpenes and oxygenated monoterpenes, along with the presence of bioactive aldehydes, suggests potential applications of *F. setifolia* essential oil in pharmacological and industrial fields.

3.2 | Antiproliferative Activity of Essential Oil

The IC_{50} values of *F. setifolia* essential oils and 5-fluorouracil (5-FU) against cancer and normal cell lines are summarised in Table 2. Statistical analysis ($p < 0.01$) reveals significant variations in the antiproliferative activity against different cell lines. The data indicate that the essential oils exhibit selective antiproliferative activity, with significantly lower IC_{50} values against certain cancer cell lines compared to normal cell lines. A2780 (IC_{50}: 8.13 μg/mL), SW1353 (IC_{50}: 20.43 μg/mL) and Calu1 (IC_{50}: 22.84 μg/mL) cell lines were found to be the most sensitive cells to the essential oils. Statistical analysis revealed that these three cell lines are part of the same significance group (group a, see Table 2), highlighting the robust and consistent antiproliferative effects of essential oils on these cancer cell lines. In contrast, the multidrug-resistant A2780ADR cell line showed a higher IC_{50} value (216.32 μg/mL, group d), indicating reduced sensitivity to the essential oils. 5-FU-like activity was observed against cell lines such as A549, MCF7 and HeLa (IC_{50}: 58.26–69.32 μg/mL, group c). Against normal cell lines (MRC5 and FL), the essential oils demonstrated significantly higher IC_{50} values (419.20 and 320.87 μg/mL, respectively, group e), suggesting lower toxicity towards normal cells compared to cancer cells. This indicates a degree of selectivity, which is a desirable property in potential antiproliferative agents.

Comparing the essential oils with 5-FU, the standard chemotherapeutic agent, reveals interesting trends. While 5-FU generally exhibited lower IC_{50} values across most cancer cell lines, the essential oils showed superior activity against specific cell lines such as Calu1, SW1353 and A2780, suggesting a potential for targeted efficacy. However, 5-FU demonstrated more consistent activity across the resistant A2780ADR cell line and normal cell lines, highlighting its broad-spectrum effects but potentially higher toxicity.

TABLE 1 | Essential oil composition of *Ferulago setifolia*.

RT	RI*	RI-lit**	Essential oils	%***
4.14	806	801	2-hexenal	0.11 ± 0.03
6.06	907	906	Thujene	0.27 ± 0.02
6.30	917	913	α-pinene	**21.58 ± 0.78**
6.74	936	935	Camphene	0.54 ± 0.02
7.57	971	973	Sabinene	**10.46 ± 0.27**
7.68	976	981	β-pinene	1.25 ± 0.03
8.17	997	1000	α-myrcene	4.05 ± 0.06
8.66	1016	1012	α-phellandrene	0.11 ± 0.02
9.11	1034	1031	α-terpinene	0.20 ± 0.03
9.32	1042	1039	1,3,5-trimethyl benzene	0.23 ± 0.04
9.41	1045	1040	*p*-cymene	0.93 ± 0.03
9.58	1052	1054	Limonene	1.79 ± 0.04
9.95	1066	1066	β-ocimene	1.59 ± 0.07
10.37	1083	1085	*Cis*-ocimene	1.88 ± 0.11
10.78	1098	1103	γ-terpinene	1.12 ± 0.05
11.15	1123	1126	*Trans*-sabinene hydrate	0.10 ± 0.04
12.01	1184	1183	α-terpinolene	0.20 ± 0.06
12.31	1206	1203	Camphenone	0.09 ± 0.01
13.60	1308	1307	α-campholene aldehyde	0.14 ± 0.04
14.43	1341	1340	Verbenol	0.22 ± 0.09
15.05	1365	1364	β-safranal	0.60 ± 0.05
15.82	1395	1392	4-terpineol	1.41 ± 0.03
19.50	1548	1553	Chrysanthenyl acetate	0.42 ± 0.05
19.70	1557	1561	Bornylene	0.37 ± 0.04
20.83	1605	1603	Lavandulyl acetate	0.41 ± 0.04
21.15	1620	1623	*cis*-*p*-mentha-2,8-dien-1-ol	0.33 ± 0.02
21.81	1650	1654	2,3,6-trimethyl benzaldehyde	**5.20 ± 0.28**
23.24	1715	1718	*m*-cymene	0.14 ± 0.06
23.64	1733	1729	2,3,4-trimethyl benzaldehyde	**41.24 ± 1.21**
26.13	1854	1856	*trans*-caryophyllene	0.19 ± 0.05
28.61	1983	1985	Germacrene-D	0.70 ± 0.04
30.21	1996	1997	γ-himachalane	0.10 ± 0.03
34.29	1981	1983	Spathulenol	0.23 ± 0.02
			Monoterpenes	46.51
			Oxygenated monoterpenes	50.27
			Sesquiterpenes	0.99
			Oxygenated sesquiterpenes	0.23
			Total identified	98.20

*Retention index determined on an HP-5MS column using the homologous series of n-hydrocarbons.
**Retention index values obtained from NIST webbook.
***Major compounds (> 5%) highlighted in bold.

The findings showed that the promising antiproliferative properties of *F. setifolia* essential oils, particularly their selective activity against certain cancer cell lines. Antiproliferative activities of extracts obtained from *Ferulago* species have been reported in various studies [27, 28]. However, the antiproliferative potential of essential oils from these species remains largely unexplored. Some essential oils with chemical compositions different from *F. setifolia* have been shown to exhibit antiproliferative effects [29, 30]. The present study is the first to investigate the antiproliferative activity of *F. setifolia* essential oils. The results not only demonstrate significant antiproliferative effects but also support the findings of previous research on the antiproliferative potential of other *Ferulago* essential oils. However, further research is needed to identify the specific bioactive compounds responsible for these effects and to explore their therapeutic potential for future pharmaceutical applications.

3.3 | Cytotoxic Activity of Essential Oil

The cytotoxicity of *Ferulago setifolia* essential oil and the standard antiproliferative drug 5-fluorouracil (5-FU) was evaluated against various cancer cell lines and normal cell lines (MRC5 and FL) at IC_{50} concentrations (Table 2). The results demonstrated that *Ferulago* essential oil exhibited significant cytotoxic effects, particularly on A2780 (33.74% ± 1.4%) and SW1353 (28.46% ± 1.2%) cell lines, highlighting its potential efficacy against ovarian and chondrosarcoma cancer cells. Compared to 5-FU, *F. setifolia* essential oil generally showed similar or higher cytotoxicity in most cancer cell lines. However, in drug-resistant cell lines such as A2780ADR, 5-FU exhibited superior cytotoxicity (24.61% ± 1.1%) compared to essential oil (15.14% ± 0.8%), reflecting the well-established effectiveness of the standard drug. Notably, the essential oil demonstrated lower toxicity toward normal cell lines (MRC5: 15.27% ± 0.8%, FL: 14.06% ± 0.9%) than 5-FU (MRC5: 17.12% ± 0.6%, FL: 16.85% ± 0.8%), suggesting a potential advantage in terms of selective toxicity. These findings demonstrate the potential of *F. setifolia* essential oil as a natural chemotherapeutic agent, with promising cytotoxic activity against cancer cells while relatively low cytotoxicity against normal cells.

TABLE 2 | Antiproliferative activity and cytotoxicity of *Ferulago setifolia* essential oil against cancer and normal cell lines.

	Antiproliferative activity IC_{50} (μg/mL)*		Cytotoxicity (%)**	
Cell lines	**EOs**	**5FU**	**EOs**	**5FU**
A549	59.54 ± 2.3^{c}	56.21 ± 2.0^{c}	27.25 ± 1.7^{b}	19.06 ± 1.6^{a}
Calu1	22.84 ± 2.1^{a}	63.81 ± 2.3^{c}	30.06 ± 1.5^{b}	22.11 ± 1.9^{a}
MCF7	58.26 ± 3.2^{c}	60.95 ± 2.4^{c}	24.26 ± 1.1^{b}	21.37 ± 1.5^{a}
A2780	8.13 ± 1.1^{a}	53.77 ± 2.0^{b}	33.74 ± 1.4^{c}	23.46 ± 1.5^{a}
A2780AD	216.32 ± 8.0^{d}	68.26 ± 2.7^{c}	15.14 ± 0.8^{a}	24.61 ± 1.1^{b}
HeLa	69.32 ± 2.6^{c}	128.75 ± 3.3^{d}	20.57 ± 1.2^{a}	23.17 ± 1.4^{a}
HT29	92.88 ± 5.5^{d}	63.05 ± 2.9^{c}	17.11 ± 0.9^{a}	22.51 ± 1.3^{a}
SW1353	20.43 ± 1.1^{a}	59.50 ± 2.3^{b}	28.46 ± 1.2^{b}	25.96 ± 1.6^{b}
Saos2	34.76 ± 1.5^{b}	64.58 ± 2.1^{c}	27.14 ± 1.5^{b}	24.68 ± 1.4^{b}
MG63	29.05 ± 1.3^{b}	69.55 ± 1.9^{c}	27.09 ± 1.5^{b}	23.01 ± 1.5^{b}
MRC5	419.20 ± 11.8^{e}	71.01 ± 2.9^{c}	15.27 ± 0.8^{a}	17.12 ± 0.6^{a}
FL	320.87 ± 7.6^{e}	62.17 ± 2.8^{c}	14.06 ± 0.9^{a}	16.85 ± 0.8^{a}

Note: $^{a-e}$Different letters indicate statistically significant differences ($p < 0.01$) within each column. Comparisons are made separately for EOs and 5-FU within antiproliferative and Cytotoxicity columns.
*IC_{50} values are given as mean values ± SDs of three independent measures.
**Percent cytotoxicity was expressed as mean values ± SDs of three independent measures.

3.4 | Antifungal Activities

The antifungal activity of *F. setifolia* essential oil was evaluated against two significant plant pathogens, *Verticillium dahliae* and *Alternaria solani*, with lethal concentration values (LC_{50}), slope of the dose–response curve and chi-square (X^2) statistics analysed to assess its effectiveness (Table 3). The LC_{50} for *V. dahliae* was determined to be 1.244 μL/Petri, indicating high sensitivity, whereas *A. solani* exhibited a higher LC_{50} of 5.42 μL/Petri, suggesting lower susceptibility. The slope of 3.492 ± 0.88 for *V. dahliae* compared to the gradual slope of 1.31 ± 0.152 for *A. solani* further showed the differential response to dose. Chi-square values of 3.785 and 2.856, respectively, demonstrate a good fit of the dose–response model for both pathogens. These findings showed the selective antifungal potential of *F. setifolia* essential oil, particularly against *V. dahliae*. The antifungal efficacy of *F. setifolia* essential oils can be attributed to their bioactive terpenes and aromatic aldehyde derivatives. It can be concluded that these essential oils exhibit significant antifungal properties, particularly due to the presence of aromatic aldehyde derivatives and bioactive terpenes such as α-pinene and sabinene, which are known for their antifungal activity.

The antifungal activity of essential oils of *F. setifolia* against plant pathogens has not been reported previously. However, in many previous studies on the *Ferulago* species, it was stated that essential oils have broad-spectrum biological activities, especially antioxidant [31], antimicrobial [16], and anti-Alzheimer [32], as well as antifungal activity against food-borne fungi [5, 33, 34], supporting our results. The significant antifungal activity observed in our study further supports this potential, highlighting *F. setifolia* essential oil as a valuable candidate for developing natural fungal control agents. Further studies on its mechanism of action and field-level applications could enhance its utility as a natural antifungal agent in sustainable agriculture.

TABLE 3 | Lethal concentration values of *Ferulago setifolia* essential oil against test microorganisms.

Microorganisms	LC_{50} (μL/Petri)	*b*	X^2
V. dahliae	1.24	3.49 ± 0.88	3.785
A. solani	5.42	1.31 ± 0.152	2.856

Note: LC_{50}: Effective dose or lethal concentration that inhibits 50% of fungal growth, X^2: (Chi-square): Goodness-of-fit statistic used to evaluate the dose–response model, including the assessment of the 95% confidence interval (Lower and Upper bounds), *b*: (Slope): Rate of change in antifungal effectiveness with increasing dose.

3.5 | Insecticidal Activity

The contact toxicity of *F. setifolia* essential oil was evaluated against *Tribolium confusum* and *Rhyzopertha dominica* at different concentrations (1%, 2.5% and 5% v/v) and exposure times (24 and 48 h) (Table 4). The results demonstrated a clear trend of increasing mortality rates with both higher concentrations and longer exposure times for both insect species. Statistical analysis (ANOVA, $p < 0.05$) revealed significant differences in mortality rates across concentrations within each exposure period. *R. dominica* was more susceptible to the essential oil, with mortality rates reaching $43.6\% \pm 0.3\%$ at 24 h and $49.3\% \pm 0.4\%$ at 48 h at the highest concentration (5%). In contrast, *T. confusum* showed lower susceptibility, with mortality rates of $31.2\% \pm 2.2\%$ at the highest concentration

TABLE 4 | The contact toxicity of *Ferulago setifolia* against *Tribolium confusum* and *Rhyzopertha dominica*.

Insect species	Concentration % (v/v)	Mortality (%) ± SE	
		24 HAT	48 HAT
Tribolium confusum	1.0	10.6 ± 2.2^{a}	13.7 ± 2.0^{a}
	2.5	16.6 ± 1.1^{a}	18.4 ± 1.4^{a}
	5.0	31.2 ± 2.2^{b}	37.7 ± 2.9^{b}
Rhyzopertha dominica	1.0	0.0 ± 0.0^{d}	0.1 ± 0.4^{d}
	2.5	22.9 ± 0.3^{b}	35.4 ± 0.2^{b}
	5.0	43.6 ± 0.3^{c}	49.3 ± 0.4^{c}
	0 (control)	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}

Note: $^{a-d}$Values by the same letter within a row are not statistically different (ANOVA $p < 0.05$, Tukey test).
Abbreviations: HAT, hours after treatment; SE, standard error.

of 5% after 24 h and 37.7% ± 2.9 after 48 h. Both of these rates were statistically higher ($p < 0.05$) than the rates observed at lower concentrations. At 2.5%, mortality rates were moderate (16.6% ± 1.1% at 24 h and 18.4% ± 1.4% at 48 h), and the lowest concentration (1%) resulted in limited mortality (10.6% ± 2.2% at 24 h and 13.7% ± 2.0% at 48 h), with no statistical difference ($p < 0.05$) between these two concentrations. At lower concentrations (1%), the mortality effect on *T. confusum* was moderate, while it was negligible for *R. dominica*. The control group (0%) showed no mortality across all treatments, confirming the toxicity of the essential oil. The findings indicate that *R. dominica* is significantly more susceptible to *F. setifolia* essential oil than *T. confusum*, particularly at higher concentrations and with longer exposure times. The statistical differences ($p < 0.05$) observed between various concentrations demonstrate the dose-dependent nature of the essential oil's contact toxicity. This suggests that *F. setifolia* essential oil holds potential for use in pest management, especially against *R. dominica*.

Essential oils derived from various *Ferulago* species have been investigated for their insecticidal activity against stored grain pests. The results indicate that the essential oils from these *Ferulago* species exhibit promising efficacy against *Ephestia kuehniella* [35], *Acanthoscelides obtectus* [36] and *Tribolium castaneum* [37], suggesting their potential application as a natural insecticide. This study is the first to evaluate the insecticidal activity of *Ferulago* species against *R. dominica* and *T. confusum*. In contrast to earlier studies, *F. setifolia* essential oils demonstrated a moderate level of insecticidal activity against two insect species, which can be entirely attributed to their chemical composition.

4 | Conclusions

The essential oil extracted from the aerial parts of *Ferulago setifolia* was analysed using GC/MS. The major constituents were identified as 2,3,4-trimethylbenzaldehyde, α-pinene and sabinene. For the first time, the biological activities of this essential oil were comprehensively evaluated. The essential oil demonstrated potent antifungal activity against *V. dahliae* and *A. solani*. Additionally, moderate insecticidal activity was observed against *R. dominica* and *T. confusum*. Furthermore, the essential oil displayed remarkable antiproliferative activity against certain cancer cell lines, showing greater effects compared to the standard chemotherapy agent 5-fluorouracil. These findings suggest that this essential oil has the potential to serve as a natural antifungal, insecticidal and anticancer agent. Overall, the results significantly improve the knowledge regarding the pharmacological and agronomic potential of *F. setifolia*.

Essential oils derived from plants have diverse chemical compositions and contain many small, nonpolar molecules that can easily penetrate cell membranes and interact with target proteins. This unique characteristic may explain their wide range of biological activities. Given these properties, essential oils show promise as natural agents for the discovery and development of antifungal, antiproliferative and insecticidal compounds. The chemical composition of plant-derived essential oils may be affected mainly by the species of source plant, geography, climatic conditions and the environment surrounded by herbivores, insects and pollinators. Therefore, this variability in the chemical composition of plant-derived essential oils emerges as a crucial factor to consider in understanding their biological activity for discovering new natural compounds. Further studies focusing on the bioactivity and therapeutic potential of these components could provide valuable insights into their functional roles and applications.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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