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Potential anthelmintic and antioxidant activities of *Jasminum* fruticans L. and its phytochemical analysis

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Abstract

Background: Ethnobotanical investigations conducted in Turkey demonstrated that Jasminum fruticans L. extract and fruit juice had been used against parasites in animals. In this study, the possible antihelmintic activity of various J. fruticans extracts contributing to its traditional use, was relatively assessed. In addition, the antioxidant potentials and phytochemical composition of the extracts were investigated since there is a relationship between helminthiasis, oxidative stress and phenolic metabolites. **Methods:** In this study, aerial parts of *J. fruticans* were subsequently extracted using *n*-hexane, ethyl acetate (EtOAc) and methanol (MeOH). In vivo anthelmintic activity of the extracts was compared with albendazole used as a reference in adult earthworms. Various methods, including free radical scavenging and metal-related activity assays, were used to assess the antioxidant capacity of the above-mentioned extracts. Assessment of phenolic composition was accomplished through total phenolic, phenolic acid, and flavonoid content assays as well as liquid chromatography-mass spectrometry (LC-MS/MS) using multiple reaction monitoring (MRM) scan modes. Further chlorogenic acid (3-O-caffeoylquinic acid) contents of extracts were quantified using high-performance thin-layer chromatography (HPTLC). Results: Between all tested extracts, MeOH extract at a quantity of 50.0 mg/mL, paralysed worms in 8.1 min and killed them in 12.8 min, showing a high anthelmintic effect similar to albendazole. Similarly, in vitro DPPH radical scavenging activity, cupric ion reduction and total antioxidant capacity experiments demonstrated that MeOH extract had significant antioxidant activity. Further phytochemical screening showed that MeOH extract was richer regarding phenolic metabolites. Chlorogenic acid, ferulic acid, caffeic acid and gallic acid were only detected in the MeOH extract. **Conclusion:** Results justify and support the use of *J. fruticans* in traditional medicine as an anthelmintic agent. Furthermore, a positive correlation was found between the strong antioxidant capacity along with the phenolic composition determined in the MeOH extract and anthelmintic activity.

Keywords: Anthelmintic, Antioxidant, Chlorogenic acid, Helminthiasis, *Jasminum fruticans* L., Oleaceae

1. Introduction

Helminthiasis is the most frequent infection caused by helminths, mainly in underdeveloped or developing countries. It occurs abundantly in diverse tropical and semitropical regions and is unfortunately categorised as a squalid tropical illness. Trichuris trichura, Ancylostoma duodenale, Necator americanus, Ascaris lumbricoides and Schistosoma spp. filarial nematodes have been reported to infect more than one billion people as a rival to AIDS and malaria¹. World Health Organization (WHO) reported that roughly one billion people globally are known to be infected with Ascariasis, 400 million people with hookworm agents, and 300 million people with Schistosomiasis². Helminths can enter the organism through the consumption of untreated drinking water or undercooked meat of infected animals, as well as through the skin wounds, mosquitoes and insect bites, even while swimming or by walking on dirty soil^{1,3}. While parasites that cause obstruction and hyperplasia in the bile duct, cause symptoms characterized by liver dysfunction in mild infections, they cause hepatitis and digestive disorders in severe infections⁴. The chance of success of anthelmintic treatment varies depending on the type of helminth, its location in the host and the number of parasites as well. While, in general, anthelmintic drugs are effective against helminths that are located in the gastrointestinal system, systemic infections involving tissues, such as Echinococcosis, Cysticercosis, Filariasis and Toxocariasis, can be partially treated with these. In such cases, surgical or other interventions may be required⁵. The drugs used in the treatment act either as helminticide or helmintifuge³. Currently, synthetic anthelmintic drugs cause gastrointestinal problems and headache as side effects⁶. Therefore, discovery of natural compounds with less side effects is important⁷. The effectiveness and lower toxicity on humans, draw attention on folkloric medicinal plants. WHO reported that 80% of the world's population prefers traditional herbal products as first line treatment. Even in modern pharmacopoeias, at least 25% of products are made from plants and many semisynthetic drugs are prepared from natural origins⁸.

There are many studies demonstrating the correlation between the oxidation mechanism of the cells and helminthiasis. Oxidative stress escalates with the amount of active oxygen species in host cells with parasitic infections. Infection of host cells via parasites is associated with higher release of free radicals, as involved in the pathogenesis of the most of the parasitic infections as well⁹. In addition, phenolic compounds with high antioxidant capacity have been used as anthelmintic in both traditional medicine and modern science.

There are investigations claiming the curative effect of polyphenols in additional or alternative treatment of helminthiasis as well as new drug lead substances¹⁰.

The genus *Jasminum* (Oleaceae) commonly grows in tropical and temperate climates and has about 200 species distributed around the world.¹¹ In ethnobotanical studies on the many species belonging to *Jasminum* genus have been used in traditional medicine as antiparasitic¹² and wound healer,¹³ in the treatment of dysmenorrhea, lepra,^{14,15} acne,¹⁶ rheumatoid arthritis and hepatitis.¹⁷ It was also used externally against eye infections,¹⁸ as an antispasmodic,¹⁹ in stopping uterine bleeding; ^{15, 20, 21} and as an oil obtained from flowers being used as a sedative in perfumery, soap industry and cosmetics industry, and aromatherapy.²²⁻²⁵ In Turkey, the infusion prepared from the flowering branches of *Jasminum fruticans* L. is internally used as a urinary enhancer and sedative²⁶, while water extract prepared from fresh branch ends is heated and used as anthelmintic²⁷. Despite the traditional utilization as an anthelmintic agent for years, there is no previous study revealing anthelmintic activity available on *J. fruticans* yet.

Phytochemical studies revealed that *Jasminum* species are rich in phenylethanoid glycosides, flavonoids, lignans saponins, sesquiterpenes and especially secoiridoids.²⁸⁻³⁰ In biological activity studies; *Jasminum* species have been found to have anti-inflammatory, analgesic, ³¹⁻³³ antimicrobial,³⁴ antiviral,³⁵ antioxidant,³⁶⁻³⁸ anthelmintic,³⁹⁻⁴³ anticancer,^{44,45} anti-obesity,⁴⁶ antiulcerogenic,⁴⁷ hepatoprotective,⁴⁸ wound healing⁴⁹ and urolithiatic⁵⁰ effects.

Although it is widely used in Turkish folk medicine, to our knowledge, *J. fruticans* efficacy in treating helminthiasis, antioxidant potential and major phenolic metabolites have not been studied in the literature. In this study, we aimed to assess the *in vivo* anthelmintic activity, antioxidant potential and phytochemical composition of *J. fruticans* considering the ethnobotanical information.

2. Material and Methods

2.1. Plant Material

Jasminum fruticans flowering branches and leaves were collected near Ballıkuyumcu village in Ankara, Etimesgut district, in the second week of May in 2018. After drying in the shade, they were powdered and used in experimental studies. The herbarium sample was determined by Prof. Dr. Hayri DUMAN from Biology Department of Gazi University,

Faculty of Science and the sample was stored in the Herbarium of Faculty of Pharmacy (Herbarium No: GUE 3462) of the same university.

2.2. Plant Extracts

To conduct chemical and biological studies, the flowering branches of J. fruticans which were dried and powdered (500 g), were extracted by maceration technique with 5 L n-hexane, ethyl acetate (EtOAc) and methanol (MeOH) for two days, respectively, and then filtered. The extracts were separately pooled and reduced to dryness in a rotavapor at 40°C. The residues were mixed with water and lyophilized. The yields of n-hexane, EtOAc and MeOH extracts were calculated as 9.27%, 23.36% and 31.19%, respectively.

2.3. Phytochemical Studies

2.3.1. Assessment of Phenolic Profile

2.3.1.1. Total phenolic content

The phenolic content of the extracts was interpreted using the spectrophotometric method of Spanos and Wrolstad.⁵¹ 0.50 g gallic acid was dissolved in 100.0 mL of 10% ethanol as stock solution. A series of solutions, where dilutions increase gradually with 0.05 mg/mL increments, in a range between 0.05-0.50 mg/mL were prepared from this stock solution. 10 mg of each test sample was completed to 10 mL with distilled water in a flask. 100 μL of both diluted stock solutions and samples were transferred into tubes. 900 μL of distilled water; 5.0 mL of Folin Ciocalteu phenol solution (1:10) and 4.00 mL of Na₂CO₃ solution (75.00 g/L) were added to each tube and left at room temperature in dark for 2h. A calibration graph was constructed after measuring the absorbance of the stock and diluted solutions at 765 nm.Then, total phenol amounts were calculated from the absorbance values of the samples using the graph.

2.3.1.2. Total flavonoid content

Total flavonoid content was measured using the spectrophotometric procedure of Woisky and Salatino 52 . After dissolving 500 μ L test sample in distilled water, 1500 μ L of 95% EtOH, 100 μ L of 10% AlCl₃ and 100 μ L of 1.00 M potassium acetate (KCH₃COO) were added and mixed. Then the total volume was completed to 5000 μ L with distilled water. After 30 min incubation at room temperature, absorbance was measured at 415 nm, using quercetin

as reference. All measurements were performed triply and the total flavonoid content of the samples was calculated by means of mg quercetin in each 1.00 g sample.

2.3.1.3. Total phenolic acid content

The total phenolic acid was measured utilizing the absorbance of the complex, formed by the reaction of hydroxycinnamic acid derivatives with the freshly prepared sodium molybdate-sodium nitrite mixture⁵³. Arnow solution was prepared through adding 50.0 mL water to 5.0 g sodium molybdate and 5.0 g sodium nitrite. 1.00 mL test samples were transferred to the test tubes and the volume of each sample was completed to 10.0 mL after adding 1.00 mL of Arnow solution, 1.00 mL of 0.1 M HCl and 1.00 mL of 1.00 M NaOH solution in it. The absorbances of test samples were measured spectrophotometrically at 765 nm. The results were calculated as the caffeic acid equivalent corresponding to 1.00 g sample. All experiments were performed triply.

2.3.1.4. Phytochemical characterization of extracts using Liquid Chromatography-Electrospray Ionization- Triple Quad Mass Spectrometry (LC/ESI-MS/MS)

Phenolic content of extracts prepared from *J. fruticans* was determined using Agilent 1260 infinity series HPLC system (vacuum degasser, a binary pump and autosample) connected to an Agilent 6420 ESI-MS/MS by using previously published method of Bardakci et al. (2020).⁵⁴ Agilent Mass Hunter software was used for acquisition and data evaluation. Standards were prepared individually and as mixtures in methanol, in a 5.0–10.0 μg/mL concentration range. For optimization of the instrumental parameters, both individual solutions and a standard mixture were used. Standard solutions were injected directly into the ion source for the optimization of ion source and MS/MS parameters. Mass range was set to 10–1200 m/z, negative ion mode to monitor single-charged ions of deprotonated phenolic compounds, [M-H]⁻. The optimized values of the ion source were: 250°C for N₂ drying gas temperature, 11 L/min for drying gas flow rate, 40 psi for nebulizer gas and 4000 V for capillary voltage.

Fragmentation of charged ions and precursor ions in MRM scanning were performed at a series of different voltages via changing capillary voltage and collision-induced dissociation voltage. In MRM scanning, the densest and most stable ion peaks of each compound were obtained for both of the precursor and product ion pairs, and then the fragmentation voltages and collision energies were re-optimized. The retention times,

optimized fragmentation voltages, collision energies and MRM transition values of the analytes are given in Table 1.

Inertsil ODS column (Zorbax Eclipse Plus 4.6×100 mm i.d., 3.5 µm particle size) from Agilent was used along with a gradient flow using mobile phase A: MeOH:H2O:Formic acid (10:89:1, v/v/v) and mobile phase B: MeOH:H2O:Formic acid (89:10:1, v/v/v)⁷¹. Flow rate was 0.40 mL/min with constant column temperature of 25 °C. The mobile phase ratio of B was increased from 0% to 10% within the first 5 min. Then it was increased to 100% within 45 min and kept at this ratio for 5 min more. The optimized injection volume was set to 10 µL, as higher volumes caused peak distortions.

2.3.1.5. High-Performance-Thin-Layer Chromatography (HPTLC)

The chlorogenic acid amounts in the extracts were measured using the HPTLC procedure of Cretu et al. 55. Standard chlorogenic acid solutions (31.25 µg/mL) and extracts were dissolved in methanol and filtered through a 0.45 µm syringe filter. Six different standard chlorogenic acid solutions with 5.0 µL of extract (2.0–8.0 µL) were analyzed triply. The sample and standard solutions were applied at a constant rate, as 8 mm bands with 10 mm gaps on silica gel coated 60 F₂₅₄ HPTLC glass plates with the Camag Automated TLC Sampler IV. The mobile phase was EtOAc:CH3COOH:CH2O2:H2O (100:11:11:27). Developments were carried out in Camag Automatic Developing Chamber (ADC-2). Humidity was controlled with ADC-2 using MgCl₂ (33% RH) for 10 min. Using Camag Derivatizer, 2.00 mL of natural product reagent (2-aminoethyl diphenylborinate) was sprayed and then heated at 100°C for 5 min using a Camag heater after 70 mm drift. Densitometric scanning was performed in fluorescence mode using Camag TLC Scanner IV and Vision CATS software after derivation. The slit size was kept at 5×0.2 mm, the micro and scan speed was set at 20 mm/s. The coefficient of variation (CV %) was observed to be below 1.00 with and the correlation coefficient (R) of the calibration curve was above 0.999. Presence of chlorogenic acid in the extracts was demonstrated via overlaying both Rf values and UV spectra obtained from extracts and standard solutions.

2.4. Anthelmintic activity

Adult earthworms (length:3-5 cm, width:0.1-0.2 cm) were used to investigate the anthelmintic activity, as they resemble round intestinal worms found in humans anatomically and physiologically. After the worms were collected from the moist soil and they were freed

of particles adhered to body surfaces washed with normal saline water. Albendazole was used as reference. ⁵⁶

Each experimental group had six worms. Test samples were dissolved in the minimum amount of dimethylformamide, and then the volume was completed to 10.0 mL with normal saline water. All test samples and reference albendazole solution were freshly prepared. Test samples and reference drug solution were poured into different petri dishes and all worms were washed in 10.0 mL normal saline water and placed in petri dishes. Experimental groups were classified as follows:

Control group: 5% dimethylformamide solution

Reference substance group: Albendazole solution (20.0 mg/mL)

n-Hexane, EtOAc and MeOH extract groups: *n*-hexane, EtOAc, MeOH extracts of flowering branches and leaves of *J. fruticans* (Concentration levels for each group: 10.0, 25.0, 50.0 mg/mL)

Throughout the experiment, the time for the worms to be paralyzed (not reviving even in normal saline water) and to die (losing its mobility and then body color) was recorded. All results were expressed as the statistical mean±S.D. of six worms in each group.

2.5. Antioxidant activity

2.5.1. Assessment of antioxidant potential depending on free radical-scavenging capacity

2.5.1.1. 2.2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging effect test

The DPPH experiment protocol Blois (1958) was applied ⁵⁷. 5000 μL (0.10 mM) freshly prepared DPPH solution was added onto 1000 μL of test samples which are prepared in various concentrations (1000-750-500-250-200-125-100 μg/ml) in methanol and vortexed for 2 min. The resulting solution was incubated at room temperature for 50 min at dark. Distilled water and butylated hydroxytoluene (BHT) were used as the blank and reference solutions consequently. The absorbance was measured at 517 nm.

2.5.2. Assessment of antioxidant potential depending on metal chelating activity

2.5.2.1. Ferric reducing antioxidant power (FRAP)

FRAP was performed according to the method by Bardakci et al. 58 . $10~\mu L$ test sample (in 1000 $\mu g/ml$ concentration) in solubilized in methanol, $30~\mu L$ distilled water and $260~\mu L$

FRAP reagent [300 mM pH 3.6 acetate buffer: 10 mM TPTZ: 20 mM FeCl₃ (10:1:1)] were placed in a 96-well plate and incubated at 37°C for 30 min. After the incubation period was completed, absorbance values were measured colorimetrically at 593 nm. As the control group, distilled water was used where BHT was reference material. The calibration curve was prepared using FeSO₄.7H₂O at different concentrations in the range of 0.0625 mM-1.00 mM. Results were calculated on FeSO₄.7H₂O corresponding to 1.00 g test sample.

2.5.2.2. Cupric reducing antioxidant capacity (CUPRAC)

Extracts of CUPRAC were measured using the method of Apak et al.⁵⁹. Using a 96-well plate, equal volumes (85 μ L) of CuSO₄, neocuproine and ammonium acetate solutions were mixed. 43 μ L test sample (in 1000 μ g/ml concentration) was added at room temperature and incubated for an hour. The calibration curve was constructed using ascorbic acid solution in the concentration range of 31.25-500.00 μ g/mL. Absorbances were measured at 450 nm. Results were calculated as ascorbic acid equivalent (AAE) of 1.00 g test sample. All experiments were conducted in triplicate.

2.5.2.3. Determination of total antioxidant capacity

The total antioxidant capacity was measured using the method of Prieto et al. 60 . 300 μ L mixture prepared with 280.0 mg sodium phosphate monobasic, 247.2 mg ammonium molybdate, 164.6 μ L sulfuric acid and 50.0 mL distilled water, was mixed in a tube with 30 μ L test sample and left to incubate at 95°C for 90 min. After the incubation was completed, the results were measured using a spectrophotometer at 695 nm. A calibration curve was created using the ascorbic acid solution in a range of 31.25-500.00 μ g/mL as a standard. Results were calculated as gallic acid equivalent (GAE) in 1.00 g test sample. All experiments were conducted in triplicate.

2.6. Statistical Evaluation

For evaluation of experiment results, the "Instat" (Windows) statistics program, including the one-way "ANOVA" test and the Students-Newman-Keuls posthoc test, were used. The statistical significance of the experimental results compared with the control and reference groups was expressed as *: p < 0.05; **: p < 0.01; ***: p < 0.001

3. Results

The success rate of anthelmintic therapy varies depending on the type of helminth, location in the host, and the number of parasites. An anthelmintic drug acts by damaging the cuticle, which causes the worm to paralyze, be partially digested or be rejected by immune mechanisms. However, most of the anthelmintic drugs may cause serious side effects and products with a lower side effect profile are needed. In this study, the anthelmintic and antioxidant activities of *J. fruticans*, which has a traditional use as anthelmintic in folk medicine, were investigated. In order to conduct chemical and biological studies, the flowering branches and leaves of the *J. fruticans* were gradually extracted with *n*-hexane, EtOAc and MeOH.

LC/ESI-MS/MS in MRM scan mode was used to determine the phenolic content. Compounds with close retention times were clearly separated from each other with the confidence of their MRM values (Figure 1; Table 2).

The results showed that *p*-coumaric acid was the major ingredient in both EtOAc and MeOH extracts. However, the presence of gallic acid, caffeic acid, ferulic acid and chlorogenic acid (3-O-caffeoylquinic acid) were detected in the MeOH extract. Unlike the MeOH extract, gallic acid and chlorogenic acid could not be detected in the EtOAc extract or remained below the LOD value. Further, spectrophotometric assays revealed that the total phenolic content of the MeOH extract was significantly higher than the EtOAc extract. The total phenol content of the *n*-hexane extract was less than the minimum required amount to be obtained, so the total phenolic substance couldn't be determined in it. The flavonoid and phenolic acid content of the MeOH extract was determined to be higher than the *n*-hexane and EtOAc extracts (Table 3).

As a result of HPTLC analysis, chlorogenic acid was 1.34% in the MeOH extract but could not be calculated in other extracts, which were consistent with the MS/MS results. The presence of chlorogenic acid in the extraction has been demonstrated by comparison of their Rf values as well as the one-to-one overlap of their UV spectra (Figures 2,3).

Antioxidant activity test results of *n*-hexane, EtOAc and MeOH extracts are presented in Table 4. The MeOH extract was found to have a strong antioxidant effect in DPPH radical scavenging activity, cupric ion reduction and total antioxidant capacity experiments, followed by EtOAc extract. Similarly, MeOH extract had a significant ferric ion reducing activity compared to EtOAc extract, whereas *n*-hexane extract had no measurable ferric ion reducing activity.

The anthelmintic activity test results showed that the time required for paralysis and death of worms was less in the MeOH extract of J. fruticans compared to the EtOAc and nhexane extract. MeOH extract caused paralysis in 8.1 min and death in 12.8 min in worms at a concentration of 50.0 mg/mL; paralysis in 15.4 min and death in 20.5 min at 25.0 mg/mL, and paralysis in 25.5 min and death in 30.7 min at 10 mg/mL. EtOAc extract caused paralysis in 11.3 min and death in 16.9 min in worms at 50.0 mg/mL; paralysis in 19.2 min and death in 23.2 min at 25.0 mg/mL, while paralysis in 22.4 min and death in 34.6 min at 10 mg/mL. n-Hexane extract caused paralysis in 14.7 min and death in 22.1 min in worms at a concentration of 50.0 mg/mL; paralysis in 30.1 min and death in 37.7 min at 25.0 mg/mL and paralysis in 51.3 min and death in 59.4 min at 10 mg/mL. Albendazole, which was used as a reference drug, caused paralysis in 6.3 min and death in 10.2 min in worms at 20.0 mg/mL (Table 5). The results showed that the MeOH extract prepared from the flowering branches of J. fruticans demonstrate very similar results to the reference drug albendazole. Anthelmintic drugs, such as albendazole, cause paralysis of worms and allow them to be eliminated via feces in humans and animals. In our study, it was determined that extracts caused both paralysis and the death of worms.

4. Discussion

Helminthiasis is a disease characterized by the localization of helminths caused by parasitic and multicellular organisms in the lumen of the gastrointestinal tract, blood and lymph vessels and some other tissues. These infections are among the most common ones in large part of the world that may cause various ailments, such as anemia, eosinophilia, pneumonia and malnutrition⁶¹. In some cases where the immune system is suppressed, the course of this disease can be very severe and fatal. In fact, it is a disease that needs to be treated because it sometimes induces a hypersensitivity reaction that leads to chronic allergic reactions characterized by anaphylaxis.¹

In the literature reviews, it's reported that gallic acid, caffeic acid, ferulic acid and chlorogenic acid, which were determined to be in high amounts in the MeOH extract in the LC/ESI-MS/MS results, have strong antioxidant effects. The potent antioxidant and DNA damage protective effects were reported for caffeoylquinic acids, dicaffeoylquinic acids, caffeic acid and its derivatives^{62,63}. The antioxidant properties of ferulic acid are related to its protective role in cellular structures and inhibition of melanogenesis⁶⁴. The antioxidant effect

of gallic acid leads to antimicrobial and immunomodulatory effects⁶⁵. In this study, the high antioxidant activity results observed in the MeOH extract was directly proportional to the amount of phenolic substances.

The aqueous extract prepared from the aerial parts of *J. polyanthum* remaining above the ground was found to have an EC₅₀ 6.41 on Teladorsagia circumcincta and the EC_{50 of} methanol extract was 14.58⁴³. The aqueous extract prepared from the *J. polyanthum* demonstrated a high anthelmintic effect and low toxicity. Ethanolic extract of J. sambac leaves was reported to cause 11.3%, 13.3% and 26.7% mortality at concentrations of 100, 200, 500 ppm on Aedes aegypti larvae, 72 hours after application⁶⁶. Lethal and paralyzing effects of the methanolic extract of the leaves of the J. mesnyi at concentrations of 20-40 mg/mL on the Eisenia fetida parasite, were equivalent to albendazole⁴¹. Chloroform, methanolic and aqueous extracts of J. grandiflorum leaves were found to have effects on the adult worm, *Pheretima posthuma* and higher anthelmintic activity compared to the reference drug albendazole, at concentrations of 20 and 40 mg/mL⁴². Ethanolic extracts of J. arborescens leaves were shown to speed up the paralysis and decrease the killing time of Pheretima posthum a^{67} . The petroleum ether extract of the above-ground parts of the J. multiflorum was demonstrated to have an anthelmintic effect on Pheretima posthuma, equivalent to piperazine citrate and albendazole, at a dose of 25 mg/mL⁴⁰. Kozan et al., showed that ethanolic and aqueous extracts prepared from the branches of the J. fruticans had strong anthelmintic effect on Syphacia obvelata³⁹.

In many preclinical and clinical studies, the total oxidant level, which is a general indicator of oxidant molecules, has been found to be high in subjects with parasitic infections. So, the effectiveness of antioxidants has been reported to be very important in strengthening the immune system and in the treatment of helminthic effects. Antioxidants contribute to the anthelmintic effect as they increase the activity of many anthelmintic drugs, such as praziquantel, and artesunate, causing severe morphological changes or parasite death during the developmental stages of the parasite 67-69.

In this study, the correlation between antioxidant capacity and anthelmintic activity was supported by chromatographic and biological studies. We highly consider that our study can contribute to the studies conducted for the development of new anthelmintic drug/drug molecules and can be regarded a resource on the way from plant to drug.

4. Conclusion

The present work exhibited that all *Jasminum fruticans* extracts showed anthelmintic activity, whereas only MeOH extract showed remarkable antioxidant activity. This might be due to the phenolic composition of the aforementioned extract. This study confirms the anthelmintic potential of *J. fruticans*, which has supported the traditional use of the aerial parts. Although the results of *in vivo* investigations revealed that the MeOH extract of *J. fruticans* might be used instead of albendazole, more studies are required to confirm its clinical effects on humans.

Credit authorship contribution statement: Esra Küpeli Akkol: Conceptualization, methodology, validation, formal analysis, investigation, data curation, writing-original draft preparation, writing-review and editing. Esma Kozan: Anthelmintic activity analysis, investigation. Hilal Bardakcı: Methodology, chromatographic analysis, review and editing. Timur Hakan Barak: Antioxidant activity, editing. Sara Khalilpour: Methodology.

Conflicts of Interest: The authors declare that they have no known competing financial interests or personal relationships that might have influenced this study.

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Table 1. Retention times, MRM transitions and optimized fragmentor voltages (FV) and collision energies (CE) of standard phenolic compounds.

| No | Compound | Molecule Weight | Retention | MRM Transition | FV | CE |
|----|------------------------|-----------------|-----------|---|-------|------|
| | | | Time | Value | | |
| 1 | Gallic acid | 170.12 g/mol | 4.67 min | $169 \text{ m/z} \rightarrow 119 \text{ m/z}$ | 110 V | 10 V |
| 2 | Catechin | 290.271 g/mol | 11.76 min | $289 \text{ m/z} \rightarrow 245 \text{ m/z}$ | 130 V | 5 V |
| 3 | Chlorogenic acid | 354.311 g/mol | 13.36 min | $353 \text{ m/z} \rightarrow 191 \text{ m/z}$ | 100 V | 10 V |
| 4 | Caffeic acid | 180.159 g/mol | 14.86 min | $179~\text{m/z} \rightarrow 135~\text{m/z}$ | 120 V | 10 V |
| 5 | Epicatechin | 290.271 g/mol | 15.50 min | $289~\text{m/z} \rightarrow 245~\text{m/z}$ | 150 V | 10 V |
| 6 | p-coumaric acid | 164.16 g/mol | 18.76 min | $163 \text{ m/z} \rightarrow 119 \text{ m/z}$ | 90 V | 10 V |
| 7 | Ferulic acid | 194.186 g/mol | 19.83 min | $193 \text{ m/z} \rightarrow 134 \text{ m/z}$ | 100 V | 15 V |
| 8 | Isoorientin | 448.380 g/mol | 20.45 min | 447 m/z →327 m/z | 170 V | 15 V |
| 9 | Vitexin | 432.381 g/mol | 21.22 min | 431 m/z →311 m/z | 130 V | 15 V |
| 10 | Luteolin-7-O-glucoside | 448.38 g/mol | 22.90 min | 447 m/z \rightarrow 285 m/z | 190 V | 20 V |
| 11 | Verbascoside | 624.592 g/mol | 23.06 min | 623 m/z →161 m/z | 210 V | 25 V |
| 12 | Leucoseptoside A | 638.619 g/mol | 23.24 min | $637 \text{ m/z} \rightarrow 461 \text{ m/z}$ | 240 V | 20 V |
| 13 | Hyperoside | 464.379 g/mol | 23.55 min | $463 \text{ m/z} \rightarrow 300 \text{ m/z}$ | 170 V | 25 V |
| 14 | Rutin | 610.521 g/mol | 23.74 min | 609 m/z →151 m/z | 210 V | 25 V |
| 15 | Rosmarinic acid | 360.318 g/mol | 24.69 min | $359 \text{ m/z} \rightarrow 161 \text{ m/z}$ | 100 V | 10 V |
| 16 | Apigenin-7-O-glucoside | 432.381 g/mol | 25.05 min | $431~\text{m/z} \rightarrow 268~\text{m/z}$ | 200 V | 30 V |
| 17 | Quercitrin | 448.38 g/mol | 25.78 min | $447~\text{m/z} \rightarrow 301~\text{m/z}$ | 160 V | 15 V |
| 18 | Martinoside | 652.646 g/mol | 26.23 min | 651 m/z \rightarrow 175 m/z | 230 V | 25 V |
| 19 | Quercetin | 302.238 g/mol | 29.08 min | $301 \text{ m/z} \rightarrow 151 \text{ m/z}$ | 150 V | 15 V |
| 20 | Naringenin | 272.256 g/mol | 29.01 min | $271~\text{m/z} \rightarrow 151~\text{m/z}$ | 140 V | 15 V |
| 21 | Apigenin | 270.240 g/mol | 32.72 min | 269 m/z →117 m/z | 150 V | 30 V |
| 22 | Caffeic acid phenethyl | 284.311 g/mol | 36.95 min | 283 m/z \rightarrow 179 m/z | 160 V | 10 V |
| | ester (CAPE) | | | | | |

Table 2. Phenolic profiling of *J. fruticans* MeOH and EtOAc extracts by LC/ESI-MS/MS.

| No | Compound/Plant | EtOAc Extract | MeOH Extract |
|----|------------------------|------------------|--------------|
| 1 | Gallic acid | - | + |
| 2 | Catechin | - | - |
| 3 | Chlorogenic acid | - | + |
| 4 | Caffeic acid | + | + |
| 5 | Epicatechin | - | - |
| 6 | p-coumaric acid | + (major) | + (major) |
| 7 | Ferulic acid | + | + |
| 8 | Isoorientin | - | - |
| 9 | Vitexin | - | - |
| 10 | Luteolin-7-O-glucoside | - | |
| 11 | Verbascoside | - | + |
| 12 | Leucoseptoside A | - | .07 |
| 13 | Hyperoside | + | + |
| 14 | Rutin | + | + |
| 15 | Rosmarinic acid | - | |
| 16 | Apigenin-7-O-glucoside | - | - |
| 17 | Quercitrin | | - |
| 18 | Martinoside | G, | - |
| 19 | Quercetin | + | - |
| 20 | Naringenin | + (trace amount) | - |
| 21 | Apigenin | - | - |
| 22 | CAPE | - | - |

Table 3. Results of the total phenolic profile of extracts prepared from *J. fruticans* (n=3).

| Material | Total Phenolic Content | Total Flavonoid Content | Total Phenolic Acid Content |
|------------------|-------------------------------|--------------------------|------------------------------------|
| n-Hexane Extract | nd | $38.67 \pm 0.49^{\circ}$ | $20.32 \pm 0.76^{\circ}$ |
| EtOAc Extract | 24.75 ± 0.98^{b} | 49.28 ± 0.85^{b} | 30.91 ± 0.49^{b} |
| MeOH Extract | 82.70 ± 1.84^{a} | 70.78 ± 1.86^{a} | 48.07 ± 1.55^{a} |

nd: no detected; *p-value: (p <0.005). a,b,c statistically significant difference (p<0.05). Results were expressed as the mean of triplicates \pm standard deviation (S.D.), as mg gallic acid equivalents (GAE) in 1 g sample for the total amount of phenolic content; as mg quercetin equivalents (QE) in 1 g sample for the total flavonoid content; re as mg caffeic acid equivalents (CAE) in 1 g sample for total phenolic acid content.

Table 4. *In vitro* antioxidant activity of extracts prepared from flowering branches and leaves of *J. fruticans* (n=3).

| Material | DPPH Scavenging Activity | Frap Activity | CUPRAC Activity | Total Antioxidant Capacity |
|------------------|------------------------------|----------------------|----------------------------|-----------------------------|
| n-Hexane Extract | $6561.37 \pm 51.89^{\circ}$ | nd | 96.63 ± 3.49^{c} | $69.25 \pm 5.96^{\circ}$ |
| EtOAc Extract | 2829.81 ± 26.22 ^b | 0.38 ± 0.002^{b} | 177.70 ± 4.81 ^b | 209.00 ± 11.16 ^b |
| MeOH Extract | 753.39 ± 15.89^{a} | 0.93 ± 0.004^{a} | 372.00 ± 8.15 ^a | 278.01 ± 15.29 ^a |

nd: no detected; a,b,c statistically significant difference (p<0.05). Results were expressed as the mean of triplicates \pm standard deviation (S.D.), and as EC₅₀ in μ g/mL equivalents for DPPH activity; as mM FeSO4 equivalents in 1 g sample for FRAP activity; for CUPRAC an total antioxidant capacity activity, and as mg ascorbic acid equivalents (AAE) in 1 g sample. 1) EC50 value of the reference compound "butylated hydroxytoluene (BHT)" in DPPH scavenging activity is found to be 368.19 \pm 10.33. 2) FRAP activity of the reference compound "butylated hydroxytoluene (BHT)" is found to be 2.26 \pm 0.18 mM FeSO4 eq. in 1 g sample.

Table 5. Comparison of the anthelmintic effects of extracts prepared from *J. fruticans* flowering branches and leaves with Albendazole.

| Test sample | Concentration (mg/mL) | Time to paralysis (min) | Time to death (min) | |
|------------------|-----------------------|-------------------------|---------------------|--|
| Control group | - | - | - | |
| | 10 | 51.3±2.0 | 59.4±1.9 | |
| n-Hexane Extract | 25 | 30.1±2.2 | 37.7±2.1 | |
| | 50 | 14.7±1.1 | 22.1±1.4 | |
| | 10 | 22.4±0.9 | 34.6±1.0 | |
| EtOAc Extract | 25 | 19.2±1.2 | 23.2±1.4 | |
| | 50 | 11.3±1.3 | 16.9±1.6 | |
| | 10 | 25.5±1.3 | 30.7±1.5 | |
| MeOH Extract | 25 | 15.4±1.0 | 20.5±1.1 | |
| | 50 | 8.1±1.1 | 12.8±1.2 | |
| Albendazole | 20 | 6.3±1.0 | 10.2±1.0 | |

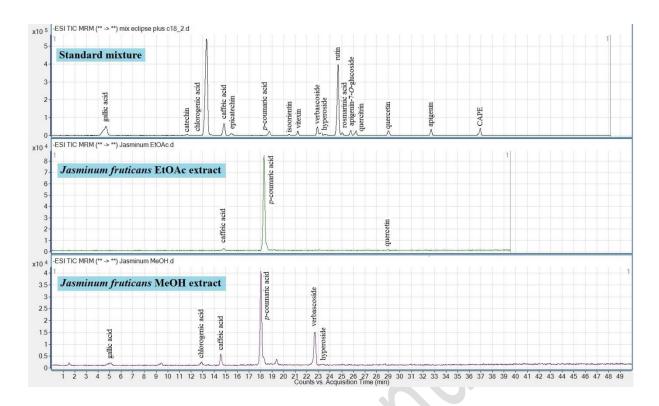


Figure 1. LC/ESI-MS/MS TIC chromatograms of the standard mixture, EtOAc and MeOH extracts of *J. fruticans*.

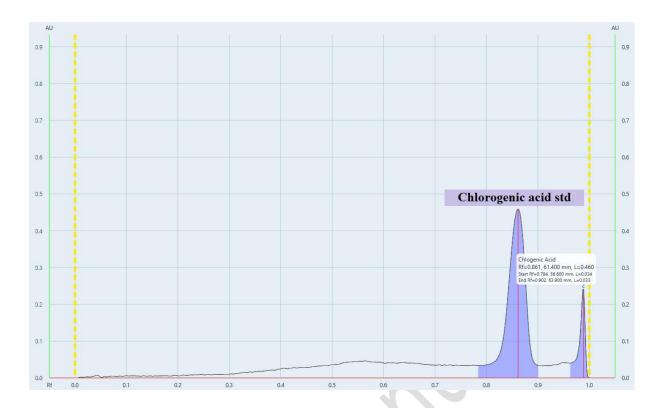


Figure 2. Densitometric chromatogram of standard chlorogenic acid and its Rf value.



Figure 3. Densitometric chromatogram of standard chlorogenic acid and *Jasminum fruticans* MeOH extract.