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Investigation of Antiparasitic Effect of *Juniperus communis* L. Fruits Extracts

Juniperus communis Meyve Ekstrelerinin Antiparaziter Etkisinin Araştırılması

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ABSTRACT

Objective: *Juniperus communis* (*J. communis*) (common juniper) is a plant that has been used for medicinal purposes for centuries. This study aims to evaluate the antiparasitic effects of ethanol, methanol, chloroform, and water extracts of *J. communis* fruits against *Plasmodium falciparum*, *Leishmania tropica*, *Trichomonas vaginalis*, and *Blastocystis*.

Methods: The antiparasitic activities of fruit extracts prepared at room temperature using the shaking maceration method were tested against *Plasmodium falciparum* using the ring stage survival test, and against *Leishmania tropica*, *Trichomonas vaginalis*, and *Blastocystis* using the broth microdilution method.

Results: The chloroform extract of *J. communis* fruits was found to be effective on *Plasmodium falciparum*, *Leishmania tropica*, *Trichomonas vaginalis*, and *Blastocystis* parasites at concentrations of 15, 10, 30 and 30 µg/mL, respectively.

Conclusion: The chloroform extract of *J. communis* fruits has shown strong antiparasitic activity against the investigated parasite species. These findings support the plant's antiparasitic potential and hold promise for future medical applications. Especially its effectiveness against metronidazole-resistant *Trichomonas vaginalis* strains is important for the development of alternative treatment options. This study highlights the potential use of *J. communis* as a medicinal plant and will contribute to the literature on research related to the isolation and structural determination of its active compounds

Keywords: Antiparasitic, *Juniperus communis*, *Blastocystis*, *Juniperus communis*, *Leishmania*, *Plasmodium*, *Trichomonas*

ÖZ

Amaç: *Juniperus communis* (*J. communis*) (adi ardıç), tıbbi amaçlarla uzun süredir kullanılan bir bitkidir. Bu çalışma, *J. communis* meyvelerinden hazırlanan etanol, metanol, kloroform ve su ekstrelerinin *Plasmodium falciparum*, *Leishmania tropica*, *Trichomonas vaginalis* ve *Blastocystis* üzerindeki antiparaziter etkilerini değerlendirmeyi amaçlamaktadır.

Yöntemler: Çalkalamalı maserasyon yöntemi oda sıcaklığında hazırlanan meyve ekstrelerinin antiparazitik aktiviteleri, *Plasmodium falciparum*'a karşı ring stage survival testi ile *Leishmania tropica*, *Trichomonas vaginalis* ve *Blastocystis*'e karşı aktiviteleri ise sıvı mikrodilüsyon yöntemi ile araştırılmıştır. Her bir parazit türü üzerinde ekstrelerin farklı konsantrasyonlarda etkinlikleri test edilmiştir.

Bulgular: *J. communis* meyvelerinin kloroform ekstresinin, *Plasmodium falciparum*'a karşı 15 µg/mL, *Leishmania tropica*'ya karşı 10 µg/mL, *Trichomonas vaginalis*'e karşı 30 µg/mL ve *Blastocystis*'e karşı ise 30 µg/mL konsantrasyonda etkili olduğu gözlenmiştir.

Sonuç: *J. communis* meyvelerinin kloroform ekstresi, araştırılan parazit türlerine karşı güçlü antiparaziter etkinlik göstermiştir. Bu bulgular, bitkinin antiparaziter potansiyelini desteklemekle birlikte gelecekteki tıbbi uygulamalar için umut vadetmektedir. Özellikle metronidazol dirençli *Trichomonas vaginalis* suşları üzerinde etkili olması, alternatif tedavi seçeneklerinin geliştirilmesi açısından önemlidir. Bu çalışma, *J. communis*'un tıbbi bitki olarak kullanım potansiyelini ortaya koymakta olup, aktivite eşliğinde izolasyon ve yapı tayini ile ilgili araştırmalar için literatüre katkı sağlayacaktır.

Anahtar Kelimeler: Antiparazitik, *Juniperus communis*, *Blastocystis*, *Juniperus communis, Leishmania*, *Plasmodium*, *Trichomonas*

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Türkiye PARAZİTOLOJİ Dergisi

INTRODUCTION

Malaria remains one of the greatest threats to human health. Among the *Plasmodium* species that cause this disease, *Plasmodium falciparum* (*P. falciparum*) and *P. vivax* are responsible for high mortality rates globally (1). The development of resistance to existing drugs necessitates the discovery of cost-effective and fast-acting new therapeutics (2-4). Similarly, leishmaniasis, a disease caused by *Leishmania* species, affects millions of people worldwide. The emergence of drug resistance in leishmaniasis treatment highlights the need for new drug candidates (5,6).

Trichomonas vaginalis (*T. vaginalis*) is a protozoan that is sexually transmitted and widely prevalent worldwide. The increasing prevalence of metronidazole-resistant strains underscores the necessity for investigating alternative treatment approaches (7).

The prevalence of *Blastocystis* ranges from 30% to 60% in developing countries. Individuals with immunosuppression and those in close contact with animals have been identified as being at particularly high risk of infection. *In vitro* and *in vivo* studies have reported its potential association with various gastrointestinal disorders and its significant role in irritable bowel syndrome (8).

Antimicrobial resistance is classified as one of the top 10 global health threats, requiring global collaboration to combat its life-threatening consequences. This situation prioritizes the discovery of new and effective antimicrobial agents. Accordingly, plant metabolites, such as terpenes, phenolic compounds, and alkaloids, have been widely studied for their antibacterial, antiviral, antifungal, and antiparasitic properties (9).

Juniperus communis (*J. communis*) (common juniper) is a plant historically used for medicinal and therapeutic purposes. In recent years, studies have investigated the antimicrobial, anticancer, and antiparasitic effects of different extracts of this plant (10).

This study aims to evaluate the antiparasitic activities of ethanol, methanol, chloroform, and water extracts of *J. communis* against various parasites, thereby exploring the potential of natural products in medical applications. The findings obtained will enhance our understanding of this plant's antiparasitic properties and guide future research.

METHODS

Ethical Approval

This research was approved by the Ethics Committee of the Faculty of Medicine, Manisa Celal Bayar University (approval date: 29/03/2023; approval number: 20.478.486/1773).

Preparation of *J. communis* Extracts

Ethanol, methanol, chloroform, and water extracts of *J. communis* fruits were obtained by the maceration technique. 5 g of ground fruits were stirred with 100 mL of solvent and macerated at room temperature for 24 hours. Each extract solution was filtered, and the solvent was evaporated under reduced pressure in a rotary evaporator until dryness. The extracts were stored at -20 °C until use.

Parasite Isolates

The parasite strains used in this study, *P. falciparum* (3D7), *Leishmania tropica* (MHOM/TR/2012/CBCL-LT), *T. vaginalis* (ATCC-50143), and *Blastocystis*, were obtained from the Parasite Bank of the Faculty of Medicine, Manisa Celal Bayar University.

In vitro Cultivation of *P. falciparum* and Screening of Extracts

The cultivation of the *P. falciparum* 3D7 strain was performed using a specialized medium prepared with 10.43 g of RPMI 1640, 25 mL of 1 M HEPES solution, 2 g of $NaHCO_3$, 0.5 mL of gentamicin solution, 0.272 g of hypoxanthine, and 5 g of albumax II.

A suspension containing 1% parasitemia was distributed into 96-well microplates. The microplates were placed in a chamber (microaerophilic incubation environment) with a gas mixture of 5% CO_2, 5% O_2, and 90% N_2. The chamber was incubated at 37 °C for 2.5 hours.

Following incubation, plant extracts were added to the parasite suspension in the microplates at final concentrations ranging from 250 µg/mL to 2.5 µg/mL, and the plates were incubated for 6 hours. After incubation, the microplates were washed, fresh medium was added, and the plates were incubated for 66 hours.

At the end of the incubation, thin smears were prepared from the microplate wells. The smears were stained with Giemsa stain and examined under a light microscope (11-14).

In vitro Cultivation of *Leishmania tropica* and Screening of Extracts

The *Leishmania tropica* isolate MHOM/TR/2012/CBCL-LT, which was isolated in Türkiye and stored in liquid nitrogen, was thawed under appropriate conditions and cultured in Novy-MacNeal-Nicolle (NNN) medium. Promastigotes grown in the NNN medium were subsequently inoculated into RPMI-1640 (Roswell Park Memorial Institute medium 1640) medium containing 10% fetal bovine serum. The growth status of the parasites was monitored every other day after inoculation. For the experiments, *L. tropica* promastigotes in the logarithmic phase, reaching a density of 10^6 promastigotes/mL, were used.

The efficacy of *J. communis* extracts against *L. tropica* promastigotes was evaluated using the CellTiter-Glo® Luminescent Cell Viability Assay (Promega, USA) kit (15-17).

In vitro Cultivation of *T. vaginalis* and Screening of Extracts

The *T. vaginalis* strain ATCC-50143 (metronidazole-resistant), stored in liquid nitrogen, was thawed under appropriate conditions and cultured in a Trypticase-Yeast Extract-Maltose medium. The growth intensity of the parasites was assessed by checking the media on consecutive days. For the experiments, *T. vaginalis* trophozoites in the logarithmic phase, reaching a density of 10^4 trophozoites/mL, were used.

The efficacy of *J. communis* extracts against *T. vaginalis* was evaluated using the microdilution method (7).

In vitro Cultivation of *Blastocystis* and Screening of Extracts

The *Blastocystis* strain, stored in liquid nitrogen, was thawed under appropriate conditions and transferred into tubes containing modified Iscove's Dulbecco's Medium supplemented with 10% inactivated horse serum. The tubes were incubated in an anaerobic environment at 35 °C. The growth status of the parasites was monitored every 2-3 days. For the experiments, *Blastocystis* parasites in the logarithmic phase, reaching a density of 10^5 parasites/mL, were used.

The efficacy of *J. communis* extracts against *Blastocystis* was evaluated using the microdilution method (18-20).

Statistical Analysis

During all *in vitro* experiments, statistical analyses were conducted to compare the effects of different *J. communis* fruit extracts (ethanol, methanol, chloroform, and water) on *P. falciparum*, *L. tropica*, *T. vaginalis*, and *Blastocystis*. The efficacy of the extracts was evaluated based on IC_{50} and LD values. Data analysis was performed using the chi-square test. All statistical analyses were done using SPSS (Statistical Package for the Social Sciences) software (version 21).

RESULTS

In vitro Screening of *P. falciparum* for Active Extracts

Examination of Giemsa-stained preparations revealed that the chloroform extract of *J. communis* fruits eliminated parasites at a concentration of 15 µg/mL. In contrast, parasites were observed in all dilutions of the other extracts.

In vitro Cultivation of *Leishmania tropica* and Screening of Active Extracts

The antileishmanial activities of *J. communis* extracts were evaluated at dilutions ranging from 250 µg/mL to 2.5 µg/mL. The IC_{50} value of amphotericin B was determined to be 0.06 µM. The IC_{50} values for the ethanol, methanol, and water extracts of *J. communis* were >250 µg/mL, while the IC_{50} value for the chloroform extract was determined to be 10 µg/mL.

In vitro Cultivation of *T. vaginalis* and Screening of Active Extracts

The IC_{50} values representing the antitrichomonal activities of the extracts were evaluated. The IC_{50} values for the ethanol, methanol, and water extracts of *J. communis* fruits were >250 µg/mL, whereas the IC_{50} value for the chloroform extract was determined to be 30 µg/mL.

In vitro Cultivation of *Blastocystis* and Screening of Active Extracts

The activities of *J. communis* extracts against *Blastocystis* were assessed at dilutions ranging from 250 µg/mL to 2.5 µg/mL. The lethal concentration (LD) values for the ethanol, methanol, and water extracts were found to be >250 µg/mL, whereas the LD value for the chloroform extract was determined to be 30 µg/mL.

Statistical Analysis

According to the chi-square test, a significant difference was observed between the chloroform extract and the other extracts in the *in vitro* efficacy against *P. falciparum*, *L. tropica*, *T. vaginalis*, and *Blastocystis* ($p<0.05$).

DISCUSSION

Juniperus species have long been used in various cultures for their antiparasitic properties (21). Similarly, the leaves and fruits of *Juniperus oxycedrus* (prickly juniper) have been applied topically to treat parasitic diseases (22). In this study, the ethanol, methanol, chloroform, and water extracts of *J. communis* were comprehensively evaluated for their *in vitro* effects on *P. falciparum*, *L. tropica*, *T. vaginalis*, and *Blastocystis*. The results indicate that this plant exhibits antiparasitic properties.

The antimalarial potential of *Juniperus* species has been particularly investigated for *J. communis*. Essential oils derived from *J. communis* have been reported to be tested against both chloroquine-sensitive and chloroquine-resistant strains of *P. falciparum*. Analyses using Gas Chromatography and Gas Chromatography-Mass Spectrometry have shown that the essential oils contain common components such as α-pinene, although the specific components vary between oils. However, it has been reported that the essential oil of *J. communis* exhibits limited antimalarial activity, with IC_{50} values exceeding 1000 µg/mL, indicating low efficacy in inhibiting parasite growth (23).

In our study, the chloroform extract of *J. communis* fruits demonstrated significant activity against *P. falciparum*. The chloroform extract exhibited a completely parasiticidal effect at a concentration of 15 µg/mL. However, the low efficacy of the ethanol, methanol, and water extracts suggests that these compounds have limited antimalarial activity.

In addition, *Juniperus procera*, a close relative of *Juniperus communis*, has been reported to exhibit notable antimalarial and antileishmanial properties. The n-hexane fraction of the ethanol extract of *Juniperus procera* was found to exhibit the most prominent activity among the tested fractions. Various diterpenes, such as abieta-7,13-diene, were isolated from this fraction, and this compound demonstrated moderate antimalarial activity against *P. falciparum* D6 and W2 clones, with IC_{50} values of 1.9 and 2.0 µg/mL, respectively. The IC_{50} values of the crude n-hexane fraction were 5.8 and 4.4 µg/mL, suggesting potential synergistic effects among the components of the extract or the presence of additional potent antimalarial compounds (24).

More comprehensive studies on *Juniperus excelsa* have revealed its significant antileishmanial effects. A study evaluating the efficacy of Greek juniper leaf and fruit extracts against leishmaniasis reported high antileishmanial activity. The petroleum ether and chloroform fractions demonstrated particularly strong activity, showing high efficacy even at low concentrations. These findings highlight the potential of Greek juniper extracts as effective antileishmanial agents (25). In our study, the high IC_{50} values of ethanol, methanol, and water extracts against *Leishmania tropica* promastigotes indicate weak antileishmanial activity for these extracts. However, the chloroform extract exhibited strong activity at a concentration of 10 µg/mL.

A comprehensive study conducted in 2013 at Shiraz University of Medical Sciences evaluated the antileishmanial activities of *Juniperus excelsa* leaf and fruit extracts, as well as leaf fractions, against *Leishmania major* in both *in vitro* and *in vivo* models. The leaf extract was reported to exhibit the highest efficacy (IC_{50}: 0.97±3.53 mg/mL), and the ethyl acetate fraction showed significant activity (IC_{50}: 1.95±5.30 mg/mL). In the *in vivo* study, a significant reduction in lesion size was observed in the test group compared to the control group ($p<0.05$), suggesting that *Juniperus excelsa* could contribute to antileishmanial therapy (26).

Additionally, a placebo-controlled clinical trial evaluated the efficacy of a topical formulation of *Juniperus excelsa* leaf extract for the treatment of cutaneous leishmaniasis. Seventy-two patients were treated with the extract in a placebo-controlled manner for three months, with cryotherapy administered as standard treatment. Weekly assessments showed that 82% of patients in the extract group achieved complete recovery, compared to 34%

in the placebo group, demonstrating a significant difference ($p<0.001$). The recovery time was also shorter in the extract group ($p=0.04$), with no major side effects reported other than mild local irritation in some patients. The researchers concluded that *Juniperus excelsa* extract is an effective adjunctive treatment for cutaneous leishmaniasis when combined with cryotherapy, increasing recovery rates and shortening recovery time (27).

In this study, the chloroform extract of *J. communis* demonstrated activity against metronidazole-resistant *T. vaginalis* at a concentration of 30 µg/mL, suggesting its potential as an alternative treatment for resistant strains. In contrast, the ethanol, methanol, and water extracts exhibited lower efficacy. Further investigation into the antitrichomonal properties of these extracts may help to elucidate the observed differences in activity.

The chloroform extract demonstrated lethal activity against *Blastocystis* at a concentration of 30 µg/mL. In contrast, the ethanol, methanol, and water extracts showed limited efficacy, even at higher concentrations, consistent with previous findings for *P. falciparum*, *L. tropica*, and *T. vaginalis* samples.

These findings highlight the diverse antiparasitic properties of *Juniperus* species, demonstrating their broad potential for medical applications. However, the effectiveness of different *Juniperus* species and their extracts varies against different parasites. While *J. communis* showed limited activity against malaria, *Juniperus procera* and *Juniperus excelsa* have shown more promising results against leishmaniasis and malaria. Further investigation of the active components in these species and the optimization of potential therapeutic applications are needed.

Future studies should focus on understanding the full spectrum of components contained within these plants and exploring the synergistic effects of these components. This will enable the development of more effective and safer antiparasitic therapies using *Juniperus* species.

Although our study comprehensively evaluated the antiparasitic activities of various *J. communis* extracts, there are some important limitations. The cytotoxic activities of the extracts have not yet been tested, which poses a limitation for their potential therapeutic applications in humans. Additionally, the efficacy of the extracts has not been tested in *in vivo* models, creating a gap in confirming their biological activity in complex systems and assessing their clinical potential.

CONCLUSION

This study demonstrates that the chloroform extract of *J. communis* exhibits strong antiparasitic activity against specific parasite species. The findings support the medicinal potential of this plant and offer promising prospects for future therapeutic applications. Notably, its effectiveness against metronidazole-resistant *T. vaginalis* strains is significant for the development of alternative treatment options. However, the low efficacy of ethanol, methanol, and water extracts highlights the need for more detailed investigations into their components and application methods. This study underscores the potential of *J. communis* as a medicinal plant and contributes to the literature for further research.

*Ethics

Ethics Committee Approval: This research was approved by the Ethics Committee of the Faculty of Medicine, Manisa Celal Bayar University (approval date: 29/03/2023; approval number: 20.478.486/1773).

Informed Consent: Since this study was conducted solely using archived parasite isolates in laboratory settings, informed consent was not required.

Footnotes

*Authorship Contributions

Concept: İ.Ç., Y.Ö., V.T., H.K., K.Y., A.Ö., Design: V.T., K.Y., A.Ö., Data Collection or Processing: Y.Ö., V.T., Analysis or Interpretation: H.K., K.Y., A.Ö., Literature Search: İ.Ç., Y.Ö., Writing: İ.Ç., A.Ö.

Conflict of Interest: No conflict of interest was declared by the authors.

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