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EVALUATION OF ANTIMICROBIAL ACTIVITY OF JUNIPER WOOD TAR (CADE OIL)

Osman Emre Özkan✉

Faculty of Forestry, Forest Industry Engineering
Kastamonu University, **Türkiye**

ABSTRACT

Juniper wood tar, obtained from the dry distillation of *Juniperus oxycedrus*, has a long history of use in both medical and industrial applications. Owing to its antibacterial, antifungal, and anti-inflammatory properties, it remains significant in the medical and veterinary fields. The tar is produced by heating wood in an oxygen-free environment at high temperatures, and its chemical composition varies depending on the wood type and distillation conditions. Medically, it is used to treat eczema, psoriasis, and acne; in veterinary medicine, it serves as an antiseptic for treating skin infections. In the cosmetics industry, it appears in shampoos, soaps, and skincare products as an anti-dandruff and antiseptic agent, while in the industrial sector, it is employed in wood preservation, paints, and leather processing. In this study, the antimicrobial properties of juniper wood tar were tested against *Staphylococcus aureus*, *Enterococcus casseliflavus*, *Enterococcus faecalis* and *Candida albicans* using the disc diffusion method. The results demonstrated significant antibacterial and antifungal activity, as indicated by the formation of inhibition zones around bacterial and fungal cultures. Moreover, the antimicrobial effect was dose-dependent, with higher concentrations producing stronger inhibition. These findings suggest the potential of juniper wood tar as a natural antimicrobial agent in the development of therapeutic treatments and preservatives. However, due to potential toxicity and skin irritation, controlled use is advised. Further research is recommended to explore its broader applications in medical and industrial contexts. Overall, this study contributes to the growing body of evidence supporting plant-derived substances as sustainable alternatives to synthetic antimicrobial agents and highlights the potential of juniper wood tar in combating antibiotic-resistant bacteria and reducing reliance on chemical preservatives.

Keywords: cade oil, antifungal, antibacterial, juniper wood

INTRODUCTION

Tar is a natural product derived from organic material such as wood, bark, roots, branches, seeds, or their combinations, with the specific raw material often determined by regional availability. During pyrolysis, biomass decomposes into three primary components: volatile substances, a water-soluble fraction, and a water-insoluble, viscous fraction commonly referred to as tar. Wood-derived tar is a dense, chemically complex mixture containing a wide range of organic compounds. It is most often obtained from coniferous species, particularly pine and juniper. *Juniperus oxycedrus*, a member of the Cupressaceae family, is native to southern Europe and the Mediterranean basin, where it grows on rocky terrains at elevations between 1700 and 3000 meters. This species has long been exploited for the production of cade oil – also known as juniper tar – which has held considerable importance throughout history. Its application spans wood

✉oeozkan@kastamonu.edu.tr, https://orcid.org/0000-0003-4011-8815

preservation, agriculture, medicine, and pharmacology (Ninich et al., 2022, 2024).

Throughout history, wood tar has served a wide range of purposes, including acting as a protective coating against moisture and microbial degradation of wood (Turkustani et al., 2019) and as an ornamental material (Burri et al., 2017; Lindborg, 2009). It has also been widely used in both human and veterinary medicine (Achour et al., 2011), particularly for the treatment of dermatological conditions (Bandaiphet and Kennedy, 2004), and as a natural insect and animal repellent (Ari et al., 2014). In Turkey, juniper tar has traditionally been employed as an antiseptic and as an ingredient in soap production (Hafızoğlu, 1994).

Tar is a chemically complex substance composed of broad groups such as resins, phenolics, polycyclic aromatic hydrocarbons (PAHs), and terpenes. Resins are primarily composed of terpenoids, while weak acids (e.g., phenols) and neutral compounds (e.g., hydrocarbons) are also present. Additional constituents may include pyrocatechol, toluene, xylene, cresol, and naphthalene. In general, tars contain aliphatic and unsaturated diterpenoid hydrocarbons that are thermally sensitive and prone to degradation. Less abundant, but still detectable, are ketones, aldehydes, and alcohol derivatives of diterpenes. Overall, aromatic hydrocarbons and resin acids are considered the principal constituents of tar (Achour et al., 2011; Ninich et al., 2022). Cade oil, in particular, is rich in aromatic hydrocarbons such as benzene, toluene, naphthalene, methylnaphthalene, and phenanthrene, along with cadinene. It also contains sesquiterpene alcohols (e.g., cadinol), phenolic compounds (e.g., cresol and resorcinol), and pyroligneous acids (e.g., acetic acid). Several cadinene isomers have been identified, including β-cadinene, δ-cadinene, and two γ-cadinene variants (Koruk et al., 2005; Ninich et al., 2022). Gas chromatography–mass spectrometry (GC/MS) analyses have further characterised its composition, identifying major constituents such as dehydroabietic acid (32.2%), abietic acid (15%), isopimaric acid (5.2%), palustric acid (5.1%), pimaric acid (3.8%), diterpene hydrocarbons (3.7%), α-terpineol (3.2%), pimaral (3.2%), and limonene (3.1%) (Hafızoğlu, 1994).

Toxicological evaluations of juniper tar have produced mixed results. In one safety assessment, undiluted juniper tar applied topically to mice and rabbits caused no signs of irritation or phototoxicity (Johnson, 2001). A dermatological study of 667 individuals with suspected contact allergies found that 28 participants (4.2%) showed sensitisation to wood tar, although only 4 (0.6%) reacted exclusively to it (Gevers et al., 2019). However, juniper tar has also demonstrated mutagenic potential in *Bacillus subtilis* and in reverse mutation assays with *Salmonella typhimurium* and *Escherichia coli* (Schoket et al., 1990). According to IFRA guidelines (2013), crude cade oil obtained via pyrolysis of *Juniperus oxycedrus* wood and branches is not recommended for use as a fragrance ingredient in end products. Only purified forms that comply with safety thresholds for PAHs are considered acceptable.

The aim of this study was to evaluate the antimicrobial activity of juniper wood tar against selected pathogenic bacteria and fungi. The disc diffusion method was employed to assess its inhibitory effects on *Staphylococcus aureus*, *Enterococcus casseliflavus*, *Enterococcus faecalis*, and *Candida albicans*. The results provide valuable evidence of juniper wood tar's potential as a natural antimicrobial agent and highlight its possible applications in pharmaceutical, veterinary, and industrial formulations.

MATERIAL AND METHODS

Material

Crude juniper wood tar was purchased from a local spice shop in Kastamonu. Nutrient Agar and Mueller–Hinton Agar were obtained from Merck (Germany). Paper discs (6 mm in diameter) were procured from Bioanalyse (Türkiye). Methanol and ethyl acetate were supplied by Sigma Aldrich (USA). Sterile plastic Petri dishes (90 mm) were sourced from Fıratmed (Türkiye).

Microorganism Strains

Antimicrobial activity was tested against selected Gram-negative (GN) and Gram-positive (GP) bacterial strains, as well as a fungal strain: *Staphylococcus aureus* ATCC 29213, *Enterococcus casseliflavus* ATCC 700327, *Enterococcus faecalis* ATCC 29212, and *Candida albicans* ATCC 10231.

Method

Antimicrobial test. The antimicrobial activity of juniper wood tar was assessed using the disc diffusion method, adapted from the protocol described by Andrews (2001). Sterile 90 mm Petri dishes, Mueller-Hinton agar, and 6 mm paper discs were used to standardise the study, following previous methods (Muğlu et al., 2019; Özdeniz et al., 2022). A solution of juniper tar was prepared by dissolving it in a 1:1 (v/v) mixture of methanol and ethyl acetate to achieve final concentrations of 5, 10, 20, 40, 100, and 200 mg/mL. From each concentration, 0.2 mL of the solution was applied to sterile, blank paper discs, resulting in discs loaded with 0.1 mg, 0.2 mg, 0.4 mg, 0.8 mg, 2 mg, or 4 mg of juniper tar. These discs were then allowed to dry for 24 hours at room temperature under sterile conditions to ensure complete solvent evaporation.

Following autoclave sterilisation, 25 mL of Mueller-Hinton agar was poured into the Petri dishes, which were then incubated at 37°C for 24 hours to check for contamination. Any contaminated plates were excluded from the study. Bacterial suspensions were prepared to match 0.5 McFarland turbidity standards (equivalent to approximately 1–2 × 10^8 CFU/mL) using a calibrated densitometer (DEN-1B, Biosan, Riga, Latvia), and were uniformly spread over the agar surface using a sterile swab. Sterile control discs and discs impregnated with the tar solutions were carefully positioned on the inoculated agar. Plates were incubated at 37°C for 24 hours, after which the diameter of the inhibition zones around each disc was measured in millimetres. All experimental procedures were conducted in triplicate to ensure reproducibility and reliability.

RESULTS AND DISCUSSION

Details of the antimicrobial activity of juniper wood tar against the tested microorganisms are provided in Table 1.

Table 1 shows the antimicrobial activity of different concentrations of juniper wood tar against four microbial strains, measured by inhibition zone diameters. *Staphylococcus aureus* exhibited steadily increasing inhibition zones from 7 mm at 0.1 mg to 22 mm at 4 mg. *Enterococcus casseliflavus* responded at all concentrations, with zones ranging from 7 mm to 12 mm. *Enterococcus faecalis* showed no inhibition at the two lowest concentrations (0.1 and 0.2 mg), but inhibition zones increased from 7.5 mm at 0.4 mg to 14 mm at 4 mg. Similarly, *Candida albicans* exhibited no inhibition at lower concentrations, with zones reaching 24 mm at the highest dose.

These results indicate that *S. aureus* is the most sensitive microorganism to juniper tar, displaying a clear dose-dependent antibacterial effect. Although *E. casseliflavus* showed smaller inhibition zones, its consistent response across all concentrations suggests a mild but sustained antimicrobial effect. The lack of inhibition in *E. faecalis* at lower doses points to a higher resistance, while the increase in activity at higher concentrations reflects a threshold-dependent response. The significant inhibition of *C. albicans* at moderate and high doses indicates strong antifungal activity. Overall, these findings demonstrate that juniper wood tar possesses broad-spectrum antimicrobial properties, with particularly strong effects against *Staphylococcus aureus* and *Candida albicans*. These

Table 1. Antimicrobial activity of juniper tar based on inhibition zone, mm
Tabela 1. Działanie przeciwbakteryjne i przeciwgrzybicze dziegciu jałowcowego na podstawie strefy zahamowania wzrostu, mm

Microorganisms	Concentration, mg					
	0.1	0.2	0.4	0.8	2	4
Staphylococcus aureus ATCC 29213	7	9	10	15	20	22
Enterococcus casseliflavus ATCC 700327	7	8	9	10	11	12
Entero*coccus faecalis* ATCC 29212	–	–	7.5	10	12.5	14
Candida albicans ATCC 10231	–	–	10	15	20	24

results are consistent with previous observations; for example, Alqahtani et al. (2020) reported significant antibacterial activity of pine tar oil against *P. gingivalis*, *P. intermedia*, and *A. actinomycetemcomitans*.

CONCLUSION

The results of this study demonstrate that juniper wood tar (cade oil) exhibits notable antimicrobial activity against both bacterial and fungal strains, particularly *Staphylococcus aureus* and *Candida albicans*. Inhibition zones observed in the disc diffusion assays confirm a dose-dependent effect, with higher concentrations producing stronger antimicrobial activity. These findings suggest that juniper wood tar has significant potential as a natural alternative to synthetic antimicrobial agents, particularly for topical applications in skin-related infections. However, due to variability in chemical composition and potential risks associated with unrefined forms – such as skin sensitisation or mutagenicity – its application should be approached with caution. Future studies should investigate the in vivo effectiveness, optimal formulations, and safety profiles of purified cade oil to support its integration into pharmaceutical, cosmetic, and veterinary products. Additionally, comparative studies involving other plant-derived tars could provide a broader understanding of their antimicrobial mechanisms and potential applications.

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OCENA AKTYWNOŚCI PRZECIWDROBNOUSTROJOWEJ SMOŁY DRZEWNEJ Z JAŁOWCA (OLEJ CADE)

ABSTRAKT

Smoła drzewna z jałowca (*Juniperus oxycedrus*) jest naturalną substancją otrzymywaną w procesie suchej destylacji. Była stosowana na przestrzeni dziejów zarówno w medycynie, jak i w przemyśle. Dzięki właściwościom przeciwbakteryjnym, przeciwgrzybiczym i przeciwzapalnym odgrywa istotną rolę w medycynie i weterynarii. Proces jej produkcji polega na podgrzewaniu drewna w środowisku beztlenowym w wysokiej temperaturze, a skład chemiczny otrzymanej smoły zależy od rodzaju drewna oraz warunków destylacji. W medycynie stosuje się ją w leczeniu egzemy, łuszczycy i trądziku, natomiast w weterynarii jako środek antyseptyczny przy infekcjach skórnych. W przemyśle kosmetycznym wykorzystywana jest w szamponach, mydłach i preparatach do pielęgnacji skóry jako substancja przeciwłupieżowa i antyseptyczna, natomiast w sektorze przemysłowym do konserwacji drewna, produkcji farb i w garbarstwie. W badaniu przetestowano właściwości przeciwdrobnoustrojowe smoły drzewnej z jałowca wobec *Staphylococcus aureus*, *Enterococcus casseliflavus*, *Enterococcus faecalis* i *Candida albicans* metodą dyfuzji krążkowej. Wyniki wykazały, że smoła ta charakteryzuje się znaczącą aktywnością przeciwbakteryjną i przeciwgrzybiczą, tworząc strefy zahamowania wzrostu wokół kultur bakteryjnych i grzybiczych. To wskazuje na jej potencjał jako naturalnego środka przeciwdrobnoustrojowego. Różne stężenia smoły dawały odmienne poziomy inhibicji, co sugeruje zależność skuteczności od dawki. Uzyskane wyniki dostarczają informacji o możliwych zastosowaniach smoły drzewnej z jałowca w rozwoju naturalnych preparatów przeciwdrobnoustrojowych i konserwantów. Ze względu na potencjalną toksyczność i możliwość podrażnień skóry zaleca się jednak jej kontrolowane stosowanie, a w przyszłych badaniach należy rozszerzyć zakres analiz nad jej wykorzystaniem w medycynie i przemyśle. Rezultaty wpisują się w nurt badań nad substancjami pochodzenia roślinnego jako zrównoważonymi alternatywami dla syntetycznych środków przeciwdrobnoustrojowych. Warto podkreślić potencjalną rolę smoły drzewnej z jałowca w zwalczaniu bakterii opornych na antybiotyki oraz ograniczaniu stosowania chemicznych konserwantów.

Słowa kluczowe: olejek jałowcowy, działanie przeciwgrzybicze, działanie przeciwbakteryjne, drewno jałowca