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In vitro Anti-amoebic and Cytotoxic Activity of *Rosa gallica* and *Picea orientalis* Leaf Aqueous Extracts

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Keywords
Acanthamoeba spp., *Rosa gallica*, *Picea orientalis*, Extract, Cytotoxicity

Abstract: *Acanthamoeba* spp. are free-living amoebae that can cause keratitis and granulomatous encephalitis in humans. This study aimed to evaluate the anti-amoebic and cytotoxic effects of aqueous leaf extracts of *Rosa gallica* and *Picea orientalis* on *Acanthamoeba* spp. and two cell lines. An environmental isolate of *Acanthamoeba* spp. was cultured on non-nutrient agar with *Escherichia coli*, and viability was tested with the trypan blue method and microscopic observation of morphologies after exposure to extracts. Cytotoxicity was tested on SH-SY5Y (human neuroblastoma) and HaCaT (human keratinocyte) cell lines via the MTT assay. No viable trophozoites were observed after six hours of incubation with 30 mg/mL of *P. orientalis* extract, whereas 20.7% of trophozoite viability was found in *R. gallica* extract with the same concentration after 24 hours. Both extracts demonstrated limited efficacy against the encysted stage, with cysts remaining viable after 24 hours, over 70% viability. In addition, dose-dependent cytotoxicity was observed for both extracts on SH-SY5Y and HaCaT cells. This study provides the first in vitro evidence of the anti-amoebic activity of *R. gallica* and *P. orientalis* aqueous leaf extracts. However, their limited effect on cysts and cytotoxicity highlights the need for further in vivo studies to explore therapeutic potential.

Rosa gallica ve *Picea orientalis* Yaprağı Su Özütlerinin In vitro Anti-amebik ve Sitotoksik Aktiviteleri

Anahtar Kelimeler
Acanthamoeba spp., *Rosa gallica,* *Picea orientalis*, Özüt, Sitotoksisite

Öz: *Acanthamoeba* spp., potansiyel patojenik serbest yaşayan amipler arasında yer almakta olup, insanlarda keratit ve granulomatöz ensefalite neden olabilmektedir. Bu çalışmada, *Rosa gallica* ve *Picea orientalis*'in yaprak özütlerinin *Acanthamoeba* spp.'ye karşı anti-amebik ve iki faklı hücre hattında sitotoksik aktivitelerinin araştırılması amaçlanmıştır. Çevresel örnekten izole edilen bir *Acanthamoeba* spp. izolatı, *Escherichia coli* ile kaplanmış, besleyici değeri olmayan agar kültüründe çoğaltılmıştır. Farklı konsantrasyonlardaki özütler ile inkübe edilen parazitin canlılığı, trypan mavisi yöntemiyle ve morfolojik değişiklikler gözlemlenerek takip edilmiştir. Ayrıca, özütlerin sitotoksik aktiviteleri, SH-SY5Y (insan nöroblastoma) ve HaCaT (insan keratinosit) hücre hatlarında MTT yöntemiyle test edilmiştir. Altı saatlik 30 mg/mL *P. orientalis* özütü inkübasyonu sonrasında canlı trofozoite rastlanmazken, aynı konsantrasyonda *R. gallica* özütü ile 24 saatlik inkübasyon sonrası trofozoitlerin ortalama %20,7'sinin canlı kaldığı gözlemlenmiştir. Trofozoit formunun aksine, her iki özütün *Acanthamoeba* spp.'nin kist formuna karşı sınırlı etki gösterdiği ve 24 saatlik inkübasyon sonrasında kistlerin %70'inden fazlasının canlı kaldığı belirlenmiştir. Ayrıca, her iki özütün SH-SY5Y ve HaCaT hücrelerinde doza bağlı değişen sitotoksik etki gösterdiği saptanmıştır. Bu in vitro çalışmanın sonucunda, *R. gallica* ve *P. orientalis* yaprağı su özütlerinin anti-amebik aktivitesine dair ilk veriler elde edilmiştir. Bu özütlerin terapötik potansiyelinin daha kapsamlı olarak değerlendirilmesi için ileri in vivo çalışmalara ihtiyaç bulunmaktadır.

1. INTRODUCTION

Acanthamoeba species are the most prevalent free-living amoebae found in both natural and human-made environments. They have been isolated from a wide range of sources, including soil, freshwater, seawater, air, humidifiers, air conditioning systems, swimming pools, contact lens solutions, surgical instruments, and medical devices such as dialysis units [1]. *Acanthamoeba* spp. has a two-stage life cycle, consisting of an active trophozoite stage and a dormant cyst stage. The trophozoite is the metabolically active, feeding, and replicating form of the parasite. The cyst stage is highly resistant and formed in response to unfavourable environmental conditions. Therefore, the parasite can survive and spread in harsh environments. From an ecological perspective, *Acanthamoeba* spp. play crucial roles in maintaining microbial balance by feeding on bacteria, which serve as their primary source of nutrition. This grazing activity gradually reduces bacterial populations, enhances nutrient cycling, and promotes soil mineralization, eventually contributing to improved plant growth [2]. *Acanthamoeba* spp. was first identified in the 1930s as a contaminant in yeast cultures. However, their clinical significance was not recognized until the 1960s and 1970s, when they were isolated from patients with granulomatous amoebic encephalitis (GAE) and *Acanthamoeba* keratitis (AK) [3]. In humans, transmission typically occurs through the inhalation of free-living amoebae via nasal passages or through skin lesions. The parasite can reach the central nervous system in two ways: via haematogenous dissemination from a primary site of infection in the lungs or skin, or direct access through olfactory neuroepithelium in the nasal cavity. *Acanthamoeba* spp. are described as opportunistic parasites capable of causing severe infections in both immunocompetent and immunocompromised individuals [4]. However, it tends to progress more rapidly and invasively in immunocompromised patients. The parasite can also cause a sight-threatening corneal infection and can result in vision impairment if left untreated. Contact lens use is the primary risk factor for AK, accounting for approximately 90% of reported cases [5]. The prevalence of AK is increasing in many countries, particularly in hospital samples, making it an emerging public health concern [6].

There is currently no standardized protocol for the diagnosis of AK. It can be isolated in cultures and detected during histopathological examination. Additionally, molecular methods such as polymerase chain reaction (PCR) offer the opportunity to detect *Acanthamoeba* DNA in suspected samples. The combined use of culture and PCR assays may help to reduce the number of misdiagnosed AK cases. In addition, the quality of the sample is crucial for diagnostic sensitivity [7]. The culture method is widely used in most laboratories due to its simplicity and its ability to support further analysis of isolates. For instance, corneal scrapings can be inoculated onto 2.5% non-nutrient agar (NNA) overlaid with a lawn of *Escherichia coli* to promote *Acanthamoeba* growth. However, the sensitivity of culture methods is generally lower compared to in vivo confocal microscopy and PCR [8]. The treatment of AK typically involves the topical application of a combination therapy using a biguanide (e.g., polyhexamethylene biguanide or chlorhexidine) and a diamidine (e.g., hexamidine or propamidine). In some cases, these agents may also be used as monotherapy [9]. However, prolonged daily use of these treatments can result in significant side effects, including corneal degeneration, glaucoma, iris atrophy, corneal ulceration, formation of cataracts, hyperaemia, and photophobia [10]. In addition to these commonly used therapeutic agents, certain antifungal drugs, such as neomycin, itraconazole, and voriconazole, have also demonstrated efficacy in the management or treatment of AK [11]. Early treatment of AK with effective drug combinations is crucial to minimize long-term ocular complications.

The Rosaceae family are rich in natural compounds with diverse biological properties and activities. The well-known species are *Rosa damascena*, *Rosa alba*, *Rosa centifolia*, and *Rosa gallica*. The biological activities of the essential oils, hydrolates, and aqueous extracts of these plants have been extensively studied in the literature [12]. Water-soluble components of *R. gallica* have demonstrated significant biological properties, showing promising results in antimicrobial, antioxidant, and bifidogenic activities [13]. Furthermore, *R. gallica* hydrolate samples were found to contain high levels of gallic and ellagic acids. The antimicrobial activity of *R. gallica* extracts has been reported against foodborne pathogenic bacteria and *Candida* species, supporting their potential as natural antimicrobials in food and pharmaceutical applications [14]. Additionally, *R. gallica* extracts were effective in controlling cariogenic bacteria in the oral microenvironment [15]. Species of the Pinaceae family naturally produce a wide range of terpenoids, which are particularly involved in the defence mechanisms of the plant. These compounds exhibit bioactive effects against various microbial and multicellular pathogens, including insects and herbivores [16]. Extracts from stem wood and bark of certain Pinaceae species have shown antimicrobial activity against a variety of human pathogens [17]. Major active components were 13-epimanool, α-cedrol, abietic acid, dehydroabietic acid, astringin, and α-terpineol. The amount of these compounds varied between root or leaf extracts of the plant. Their antimicrobial activity and chemical composition have offered good potential for applications in pharmaceuticals and food preservation [18].

Many plant-derived products have been tested on *Acanthamoeba* spp. [19]. However, to the best of our knowledge, there was no study in the literature dealing with the anti-amoebic activity of *R. gallica* and *P. orientalis*. Plant-based compounds and extracts may offer promising potential for the development of novel therapeutic agents for the treatment of *Acanthamoeba* spp. infection in humans. The present study aimed to evaluate the anti-amoebic and cytotoxic activities of aqueous leaf extracts of *R. gallica* and *P. orientalis* on *Acanthamoeba* spp. and two different human cell lines.

2. MATERIAL AND METHOD

2.1. Isolate and Cultivation

Acanthamoeba spp. AduA1 isolate was used in the experiments. It was originally isolated from an environmental water sample and cultured on non-nutrient agar (NNA) medium. The isolate was defined as *Acanthamoeba* spp. by partial amplification of the 18S rRNA coding gene followed by BLAST analysis in GenBank (Acc. No. PQ651434). The culture medium was prepared as follows: a Ringer tablet (Merck) was dissolved in distilled water according to the manufacturer's instructions and autoclaved at 121°C for 15 minutes. Bacteriological agar was then added at a final concentration of 2.5%, re-autoclaved, cooled, and poured into Petri dishes to solidify. Finally, the dishes were covered with a lawn of *Escherichia coli* (ATCC 25922). The isolate was cultivated at 30ºC in a standard bacteriological incubator.

2.2. Preparation of Plant Extracts

Leaves of gallic rose (*Rosa gallica*) and Caucasian spruce (*Picea orientalis*) were collected in Gaziantep, Turkey (36°58'43.1"N and 37°18'04.6"E). A mixture of 200 mL ultrapure water and 100 g of leaf was prepared in 1000 mL Erlenmeyer. The mixture was boiled under continuous stirring using a magnetic stirrer for 2 hours. After cooling to room temperature, it was filtered using a funnel. The resulting filtrate was lyophilized, and the precipitated extract was stored at -20 °C until use [20].

2.3. Determination of Anti-amoebic Activities

Acanthamoeba spp. trophozoites were harvested from NNA medium two days after inoculation. The medium was overlaid with 10 mL of sterile phosphate-buffered saline (PBS), and trophozoites were scraped from the surface using cell scrapers (Corning®). The resulting suspension was centrifuged at 3000 × g for 5 minutes, and the final concentration was adjusted to 1 × 10^4 cells/mL using sterile PBS. *Acanthamoeba* spp. cysts were collected from two-week-old NNA cultures and prepared using the same method as for the trophozoite forms.

The viability of *Acanthamoeba* spp. trophozoites and cysts were observed for 24 hours (after one, 3, 6, 12, and 24 hours of incubation). The final concentrations of plant extracts were adjusted to 1, 5, 15, and 30 mg/mL. After each time interval, 25 µL of the sample was mixed with an equal volume of trypan blue (Biochrome, 0.5%) and immediately examined under a light microscope at 40× magnification. The viable cells (unstained) and non-viable cells (stained or morphologically degenerated) were counted [21]. The percentage of cell viability was calculated using the following formula: 100 X viable cells/ (viable + non-viable cells). Three samples were taken for each observation time, and the mean percentage of cell viabilities was calculated. The experiments were performed at room temperature.

2.4. Cell Cultures

Human keratinocytes (HaCaT) and human neuroblastoma (SH-SY5Y) cells were cultured in DMEM medium supplemented with 10% foetal bovine serum, 1% penicillin-streptomycin and HEPES. Cells were maintained in 75 cm2 culture flasks, with the medium renewed every two days. When cell confluence reached approximately 90%, the cells were passaged and subsequently used in cytotoxicity experiments [22].

2.5. Determination of Cytotoxic Activities

SH-SY5Y and HaCaT cell lines were seeded in 96-well plates at a density of $5x10^3$ cells per well. The cells were incubated for 24 hours in a CO_2 incubator for adherence to the bottom of the wells. Following incubation, the cells were treated with the extracts at concentrations of 1, 10, 100 and 1000 µg/mL for 24 hours. The logarithmic doses are typically used for to created dose–response curves in cytotoxicity studies. In the present study, a wide dose range of the extracts (1–1000 µg/mL) were tested on human cell lines. Subsequently, 10 µL of MTT dye (5 mg/mL) was added to each well. After 4 hours of incubation, the medium was removed, and the resulting formazan crystals were dissolved in 100 µL of DMSO. Absorbance was measured at 570 nm using a plate reader. The percentage cell viability was calculated using the following formula: (OD test sample/OD control) X 100 [23].

2.6. Statistical Analysis

Statistical analyses were performed using GraphPad Prism version 9.3. The measurements were repeated three times, and results were presented as mean ± standard deviation (SD). Differences between groups were determined using the one-way ANOVA test. Statistical significance levels were set as follows: $p < 0.05$; $p < 0.01$; $p < 0.001$; $p < 0.0001$.

3. RESULTS

3.1. Anti-Amoebic Activity of Plant Extracts

The anti-amoebic effects of the extracts were evaluated separately on the trophozoite and cyst forms of *Acanthamoeba* spp. Overall, the cyst forms were more resistant to the extracts compared to the trophozoite forms. After six hours of inoculation with 30 mg/mL of *P. orientalis* extract, all trophozoites were non-viable (Figure 1). In contrast, viable trophozoites were still observed after 24 hours of exposure to *R. gallica* extract (Figure 2). Cyst forms remained over 72% viable after 24 hours of treatment with both of the plant extracts (Figure 3). The time and dose-dependent activities of the extracts were presented in Figure 4 for *R. gallica* and Figure 5 for *P. orientalis*. The viability of trophozoites decreased significantly at concentrations of 5 mg/mL, 10 mg/mL, and 15 mg/mL ($p < 0.05$, $p < 0.0001$, and $p < 0.0001$, respectively) after 24 hours of treatment with *R. gallica*, however, no statistically significant decrease was observed in cyst viability over the same period ($p > 0.05$).

In contrast, the viability of trophozoites treated with *P. orientalis* for 24 hours decreased significantly at concentrations of 10 mg/mL and 15 mg/mL ($p < 0.0001$). Additionally, a statistically significant decrease in cyst viability was observed only at 30 mg/mL ($p < 0.05$) after 24-hour treatment with *P. orientalis*.

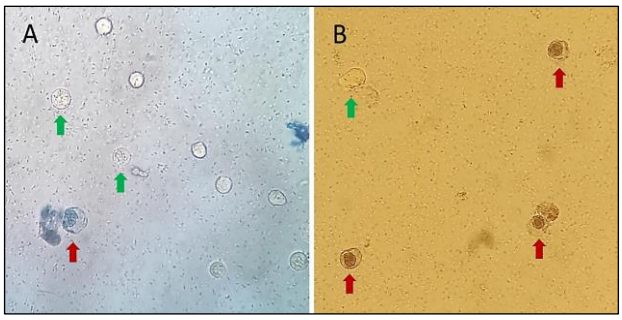

Figure 1. Effect of *R. galllica* aqueous extract on *Acanthamoeba* spp. trophozoites. Images display viable (green arrows) and non-viable (red arrows) trophozoites of *Acanthamoeba* spp. following treatment with 30 mg/mL of the extract: (A) after one hour of incubation and (B) after 24 hours of incubation.

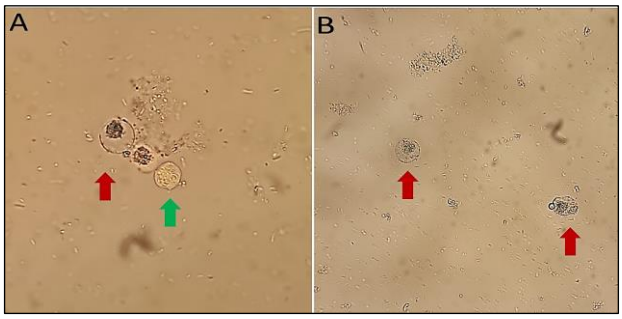

Figure 2. Effect of *P. orientalis* aqueous extract on *Acanthamoeba* spp. trophozoites. Images display viable (green arrows) and non-viable (red arrows) trophozoites of *Acanthamoeba* spp. following treatment with 30 mg/mL of the extract: (A) after 3 hours of incubation and (B) after 6 hours of incubation.

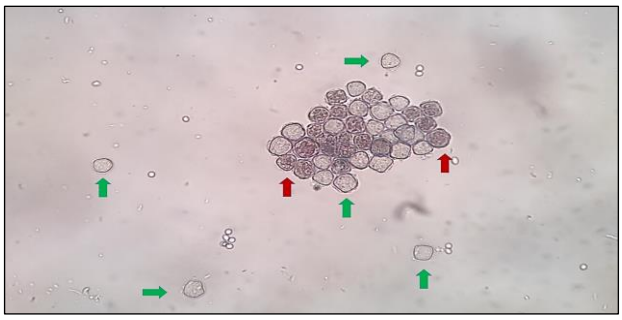

Figure 3. Viability of *Acanthamoeba* spp. cysts following treatment with 30 mg/mL of *P. orientalis* aqueous extract after 3 hours, viable cysts (green arrows) and non-viable cysts (red arrows).

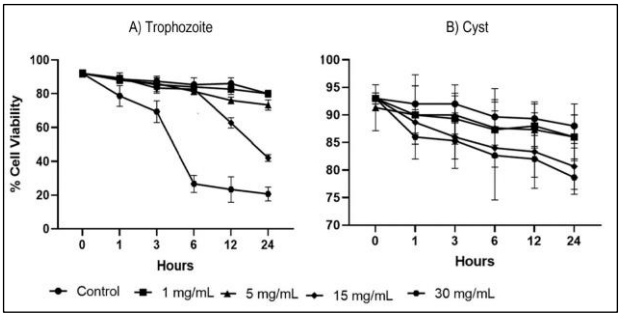

Figure 4. Graph showing the anti-amoebic activity of *R. gallica* aqueous extract on *Acanthamoeba* spp.: (A) trophozoite form and (B) cyst form.

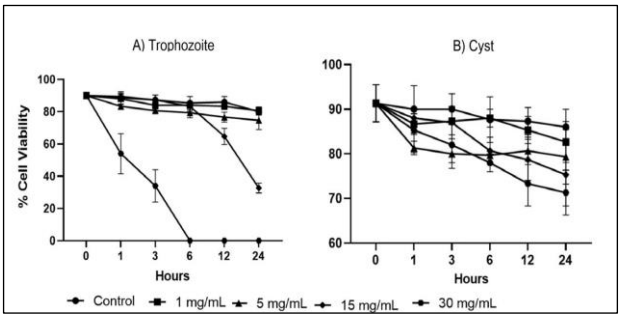

Figure 5. Graph showing the anti-amoebic activity of *P. orientalis* aqueous extract *Acanthamoeba* spp.: (A) trophozoite form and (B) cyst form.

3.2. Cytotoxic Effect of Plant Extracts

The cytotoxic effects of two aqueous extracts at concentrations of 1, 10, 100 and 1000 µg/mL on SH-SY5Y cells were evaluated by the MTT method. After 24 h of incubation, it was found that the cytotoxic effects of both aqueous extracts increased dose-dependently in both cell lines. The % cell viability rates of SH-SY5Y and HaCaT cells treated with 1000 µg/mL of *R. gallica* extract were found to be 31.27±1.62 and 25.04±0.04, respectively ($p<0.0001$) (Figure 6A and 6B). The % cell viability rates of SH-SY5Y and HaCaT cells treated with 1000 µg/mL *P. orientalis* were calculated as 33.18±8.84 and 34.30±7.61, respectively ($p<0.0001$) (Figure 6C and 6D).

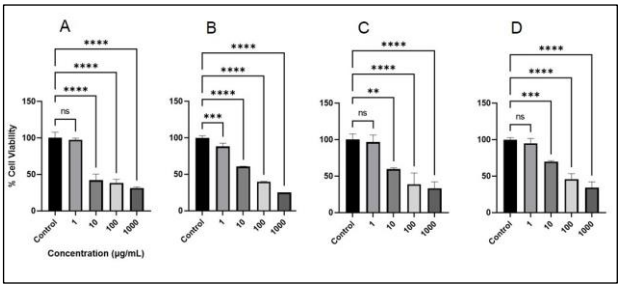

Figure 6. Graph showing the cytotoxic activities of plant extracts: (A) *R. galllica* on SH-SY5Y cells, (B) *R. galllica* on HaCaT cells, (C) *P. orientalis* on SH-SY5Y cells, and (D) *P. orientalis* on HaCaT cells. Statistical significance levels: *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; ****, $p<0.0001$.

4. DISCUSSION AND CONCLUSION

Acanthamoeba spp. are a common group of free-living amoeba in the environment and water sources. These free-living amoebas can act as opportunistic parasites, causing sight-threatening keratitis and life-threatening encephalitis in humans. Accurate and early diagnosis is crucial for identifying the causative agent and differentiating it from other pathogens. Effective treatment is essential to prevent long-term clinical complications. In recent years, resistance of *Acanthamoeba* spp. to conventional therapies and chlorine has been reported, highlighting the need for alternative therapeutic options, including plant-derived compounds and chemical agents [24, 25].

In the present study, we tested the anti-amoebic activity of *R. gallica* and *P. orientalis* leaf aqueous extracts against both the trophozoite and cyst forms of *Acanthamoeba* spp. The extracts showed a time- and dose-dependent effect on the parasite. To the best of our knowledge, reviewing the literature, no previous studies have investigated the amoebicidal activity of these two extracts. The extract of *P. orientalis* exhibited rapid and effective amoebicidal activity, with complete trophozoite mortality observed after six hours of exposure at a concentration of 30 mg/mL. However, *R. gallica* extract did not achieve total trophozoite mortality even after 24 hours. In addition, morphological changes caused by the two extracts differed, with *P. orientalis* extract causing more disruptions. The cyst forms were highly resistant to both extracts, with over 70% remaining viable even after 24 hours of incubation. The resistance of *Acanthamoeba* spp. cysts were probably related to the double-walled structure and reduced metabolic activity, making them less susceptible to many therapeutic agents [26]. It was reported that the colour and contrast of stains vary according to the developmental stages of free-living amoebas including *Acanthamoeba* spp. and *Naegleria* spp. [27]. Since cysts at different stages of maturation may differ in dye uptake, distinguishing immature and non-viable cysts can be challenging. To minimize this variability, two-week-old NNA cultures were used in the present study to ensure a more consistent level of cyst maturation, and morphological changes were also documented.

The differences in the anti-amoebic activities of the two extracts are likely due to variations in their bioactive components. Studies have reported that rose extracts exhibit diverse biological effects, including antimicrobial and antioxidant properties [28]. The composition of rose plant extracts includes high amounts of polyphenols, anthocyanins, and flavonoids [29]. Another study identified phenethyl alcohol, β-citronellol, and geraniol as the major chemical components in rose water samples, with a total of 22 different constituents detected [30]. The chemical composition of rose plant leaf has been extensively studied, revealing the presence of flavonoids, hydroxycinnamic acids, phenols, tannins, terpenes, aldehydes, alcohols, minerals, fatty acids, and vitamin C. These components contribute to various biological functions, such as diuretic properties and applications as natural dyes [31]. Rose aromatherapy is a widely recognized traditional approach, used to treat dysmenorrhea, stress, depression, and anxiety. In addition to its therapeutic effects, the antimicrobial properties of rose extracts against various microorganisms have been documented. The growth of several bacteria was significantly inhibited by rose extract [32]. Different parts of the rose plant exhibit distinct chemical compositions and biological activities. Rose hip extracts, for example, have shown potential antimicrobial properties, with *R. rugosa* fruit extract exhibiting the highest antimicrobial activity against *Staphylococcus* spp. [33]. A study demonstrated antimicrobial and antioxidant activities of methanol extract of *R. gallica var. aegyptiaca* against a range of microorganisms [14]. The antimicrobial activity of extract varied depending on the extraction method including hexane, methanol, hydromethanol 80%, chloroform, and water. The hydromethanol method showed the highest antimicrobial activity. Essential oils derived from *R. damascena* exhibited promising fungicidal and antibacterial activity [34]. The diverse chemical compositions and biological activities of different rose plant parts and their extracts have been highlighted in the literature, contributing to applications for a wide range of therapeutic and antimicrobial purposes. A comprehensive study from Turkey investigated the chemical components and antimicrobial activity of *P. orientalis*. They reported limonene, β-pinene, and α-pinene as the major components. In addition, monoterpene hydrocarbons were abundant in essential oils in resin. A total of 22 microorganism species were tested with the agar well diffusion method and the highest antibacterial activity was noted against *S. typhimurium* and *S. aureus*. In addition, antifungal activity was highest for *C. albicans* [35]. In contrast, another species of *Picea* (*P. abies*) did not show any promising antimicrobial activity on both *E. coli* and *S. aureus* [36]. A study reported that the major components of *P. abies* wood and bark extracts were di-hydroxyverrucosane and larixol. The extracts also had antimicrobial activities against a variety of human pathogen, foodborne, and agricultural microorganisms [37].

A variety of plant-derived materials has been tested for their anti-amoebic activity against *Acanthamoeba* spp., with some studies reporting promising results. For example, a study evaluating 200 plant extracts from South Asia identified three with significant amoebicidal activity [38]. Because of its geographical location, plant biodiversity in Turkey is very high. Previous studies in Turkey have identified several plant extracts with different levels of anti-amoebic activity against *Acanthamoeba* spp., including extracts from *Allium species, Ceratophyllum spp.,* and *Pastinaca* spp. [39-41]. Consistent with our findings, most studies also observed that the cyst forms of *Acanthamoeba* spp. were more resistant to plant extracts than the trophozoites.

Cell cytotoxicity refers to the ability of a substance to cause damage to cells, leading to cell dysfunction or death. It is an important parameter in toxicology, pharmacology, and cancer research [42-44]. Plant extracts

can be used as supportive agents in cancer treatment to minimize the side effects of chemotherapy due to their cytotoxic phytoconstituents [45]. The extract of *R. gallica* has been reported to contain cytotoxic components such as glycyrrhetinic acid, hyperoside, caffeic acid, quercetin and ferulic acid [46]. At the highest concentration (1000 µg/mL) of *R. gallica* extract, the viabilities of SH-SY5Y and HaCaT cells were 31.3% and 25%, respectively. In addition, at the same concentration of *P. orientalis*, the cell viabilities were 33.2% and 34.3%, respectively. It was reported that the cytotoxic activity of rose extracts was directly dependent on the extraction method, the concentration, and the target cell. For example, the sensitivity of normal cells from animals and humans, as well as cancer cell lines were different after being treated with aqueous extracts of Iranian rose [47]. The cytotoxic activity of the aqueous extract differed between human HeLa tumour cells and human lymphocytes. Lower concentrations of the extract were effective against cancer cells, while higher concentrations were required to affect healthy human cells. Another study investigated the effects of both aqueous and ethanolic extracts of *Rosa damascena* on human gastric cancer cells [48]. Although all concentrations significantly reduced cell viability, the ethanolic extract exhibited a stronger inhibitory effect than the aqueous extract, probably due to differences in chemical composition. The IC_{50} values of the ethanolic and aqueous extracts were 2.517 µg/mL and 3.887 µg/mL, respectively. Hydrosols from Bulgarian rose flowers, extracted by water–steam distillation using a semi-industrial process, exhibited low cytotoxic and genotoxic effects in cell-based assays, suggesting their potential for safe human use [49]. The cytotoxic activity of aqueous extracts from spruce bark (*P. abies*), via classical water bath extraction, was tested on tumour and non-tumour cell lines. These extracts were not cytotoxic on the human keratinocyte cell line and stimulated the proliferation of the cells [50]. Essential oils from another *Picea* species demonstrated strong anti-tumour activity against MCF-7 breast cancer cells [51]. Additionally, a hot water extract of black spruce (*P. mariana*) showed promising antioxidant and anti-inflammatory properties [52].

Although our study was designed as an in vitro study, it provides the first data on the anti-amoebic effects of *R. gallica* and *P. orientalis*. The extracts show varying potential as anti-amoebic agents, particularly against trophozoites. However, their efficacy against cyst forms requires further investigation to test their therapeutic efficiencies. Future research on in vivo effects, toxicity assessments, and the development of standardized formulations for clinical applications will be valuable.

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