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STIMULATORY EFFECTS OF RAW *CHELIDONIUM MAJUS* EXTRACTS ON THE GROWTH OF VARIOUS STRAINS OF MICROORGANISMS

Teodora-Maria BARNEA[1,2], Constantina Bianca VULPE[1,3,*], Oana Roxana TOADER[1,2], Corina DUDA-SEIMAN[4], Vasile OSTAFE[1,2], Gheorghita MENGHIU[1,2]

[1]Advanced Environmental Research Laboratories; West University of Timisoara, Oituz 4A, 300086 Timisoara, Romania

[2]Department of Biology; Faculty of Chemistry, Biology, Geography, West University of Timisoara, Pestalozzi 16, Timisoara 300115, Romania

[3]Department of Scientific Research in Biology, Advanced Environmental Research Institute, West University of Timisoara, Oituz, 4, 300086 Timisoara, Romania

[4] Department of Cellular and Molecular Biology, Faculty of Medicine, Titu Maiorescu University, Vacaresti 187, 031593 Bucharest, Romania

**Corresponding author's e-mail: constantina.vulpe@e-uvt.ro*

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ABSTRACT

Chelidonium majus is a plant found mainly in Europe and Asia, traditionally used in therapeutic practices. It is characterized by small leaves and yellow flowers. While alcoholic extracts of Chelidonium majus are known for their antimicrobial properties, there is limited scientific evidence on the efficacy of aqueous extracts. In this study, the effects of crude aqueous and alcoholic extracts of Chelidonium majus on microbial strains including Escherichia coli, Staphylococcus aureus, Enterobacter cloacae and Candida albicans were evaluated. Plant material was collected from two locations in Romania: the city of Timisoara (a heavily polluted urban area) and the rural commune of Bacia, Hunedoara County (a less polluted area). The collections were performed in different seasons (spring, summer, autumn, and winter) to assess whether environmental pollution levels and seasonal variations influence the biological activity of the extracts. The extracts were prepared by homogenizing 50 g of plant tissue in 50 mL of distilled water or 50% ethanol for 5 minutes. These extracts were then diluted to different concentrations using strain-specific media, inoculated with 2 µL of preculture and incubated in microtiter plates for 20 h at 30°C with shaking at 500 rpm. Optical density at 600 nm was measured before and after incubation and microbial growth rates were calculated accordingly. The results showed that increasing concentrations of aqueous extracts resulted in a significant increase in both optical density and microbial growth rate, indicating a stimulatory effect on the proliferation of microorganisms. In contrast, alcoholic extracts caused a concentration-dependent decrease in optical density and growth rate, suggesting an inhibitory effect. Viability tests further confirmed the

bacteriostatic and fungistatic activity of alcoholic extracts. These findings highlight the critical importance of the extraction method in determining the biological activity of Chelidonium majus preparations. Antimicrobial effects were mainly associated with ethanol-based extraction, confirming that the choice of solvent significantly influences the recovery and efficacy of bioactive compounds. Furthermore, the inclusion of different locations and seasons as experimental variables show the potential role of environmental and temporal factors in modulating the bioactive properties of the plant.
KEY WORDS*:* *greater celandine, bacteria, yeast, aqueous extracts, alcoholic extracts.*

INTRODUCTION

Chelidonium majus has been known since antiquity, being mentioned in the works of Paracelsius, Dioscorides, Pliny. Dioscorides calls it "swallow grass", because it blooms when the swallows arrive in May and withers when they leave for warmer countries (Dumitriu et al., 2022). It belongs to the family *Papaveraceae*, which also includes plants such as Canada poppy (*Sanguinaria canadensis* L.), Persian poppy (*Papaver bracteatum* Lindl.) or Opium poppy (*P. somniferum*). *C. majus* is a short-lived hemicryptophyte species (Zare et al., 2021). The plant is characterized by a stem that can reach heights of up to one metre, is branched and sporadically covered with trichomes. It has traditionally been used in Western herbal medicine to treat liver disease, gastric ulcers, oral infections, aches and pains, skin rashes and tuberculosis. Externally, the plant's latex has long been known as a folk remedy for the removal of warts, the healing of old and persistent skin ulcers, and the treatment of corneal opacities (Gilca et al., 2010; Samatadze et al., 2020). The leaves of *C. majus* are arranged alternately. The basal leaves have a glaucous, bluish-green hue due to a fine waxy coating at the base and bright green toward the tip. The basal leaves, with long petioles, have an obovate outline and are pinnately sectate, with segments bearing 5 to 7 lobes. In contrast, the apical leaves have short petioles and consist of 3-lobed leaflets. Between April and October, *C. majus* develops umbellate inflorescences bearing 2 to 6 flowers, each with four yellow petals and two white sepals that fall prematurely. The fruit of the plant is a podiform, elongated capsule, about 3 cm long, dehiscent, with two valves and containing shiny, oval, dark brown or black seeds equipped with lipid-rich structures that attract ants for seed dispersal. The plant's root system consists of a short taproot, from which numerous elongated lateral roots grow. The plant is also distinguished for its latex, which varies in colour from yellow to orange.

The healing capabilities of *C. majus* are attributed to its various biologically active components. In terms of composition, this plant is rich in isoquinoline alkaloids, ranging from 0.27 - 2.25% in the aerial part and 3-4% in the roots. So far, more than 70

chemicals have been discovered and identified in *C. majus*, including alkaloids, flavonoids, saponins, vitamins (such as vitamin A and C), mineral elements, sterols and various acids and their derivatives (Zielińska et al., 2018). Detailed phytochemical studies have revealed a wide range of alkaloids in different sections of the plant, such as benzophenanthridines. In total, five main categories of alkaloids have been classified in *C. majus*, all of which are phenanthridine derivatives. Among the key alkaloids present in *C. majus* are chelidonine, berberine, coptisine, sanguinarine and chelerythrin, found in various portions of the plant (Nile et al., 2021; Dumitriu et al., 2022; Zhou et al., 2012). *C. majus* orange latex contains a large number of isoquinoline alkaloids, at least 20 have been isolated and chemically identified. Isoquinolines represent the largest class of alkaloids, with over 2 000 distinct structures identified to date, many of which hold significant importance as plant-derived pharmaceuticals. Over the centuries, the plant's orange-yellow latex has been used in traditional treatments to combat warts and symptoms associated with human papilloma virus (HPV) infections (Zielińska et al., 2019). Although its antiviral and antitumor efficacy was initially attributed to the presence of alkaloids, more recent research suggests that proteins in the latex composition may also play a crucial role in its therapeutic effects (Musidlak et al., 2022). In other studies, four alkaloids (sanguinarine, chelidonine, protopine, stilopine) from *C. majus* latex were evaluated against melanoma cells, leading to apoptosis of cancer cells with only a mild effect on normal cells. Multiple studies have demonstrated the efficacy of *C. majus* latex sap on dermal tumors, and clinical data belonging to the research proved the destruction of pathologically altered tissues and eradication of preneoplastic lesions after regular application of latex (Gracz-Bernaciak et al, 2021; Rogelj et al, 1998; Zielińska et al., 2020).

In recent years, research conducted on *C. majus* extract and its bioactive compounds has revealed a vast therapeutic potential, demonstrating antimicrobial, liver-protective, anticarcinogenic, antioxidant, Alzheimer's disease, immune regulating, anti-inflammatory and analgesic effects in both *in vivo* and *in vitro* studies (Amal & Pratim, 2015; Monavari et al., 2012; Segneanu et al., 2024; Stickel et al., 2009). Thus, *C. majus* and its compounds offer new and exciting perspectives for the research and treatment of various diseases (Tuzimski et al., 2023). The total alkaloid extracts of *C. majus* have shown antiviral activity against several types of viruses. The ethanolic extract of *C. majus* inhibits the growth and development of Herpes simplex virus type 1 (HSV-1) (Monavari et al., 2012). Various components of *C. majus* have been incorporated into numerous traditional and homeopathic medicinal preparations (Benninger et al,, 1999; Moro et al., 2009). In homeopathy, certain dilutions of *C. majus* extract are used to

address a wide range of diseases. There are already homeopathic and herbal products on the market that include *C. majus*, including Gastol, a phytotherapeutic remedy traditionally used to support liver function and bile secretion (Amal & Pratim, 2015). Along with other plants, *C. majus* possesses anti-inflammatory and antioxidant qualities, and the presence of alkaloids and flavonoids in this plant gives it a powerful antioxidant status (Dumbravă et al., 2008; Heo et al., 2013; Jakovljevic et al,, 2013). These antioxidants are essential in fighting free radicals in the body and have a significant impact in reducing eczema (Khodabande et al, 2017). Due to its antibacterial properties, *C. majus* has been employed in the treatment of various dermatological conditions, including those associated with both Gram-positive and Gram-negative bacterial infections. Positive therapeutic outcomes have been reported in cases such as acne and other common skin disorders (Krzyżek et al., 2021). Chelidonine and homochelidonine exhibit similar effects to morphine, with properties to suppress myocardial function, induce narcotic effects and calm the central nervous system. They also relax smooth muscle in the coronary arteries and large blood vessels (Krahulcová, 1982; Krizhanovska et al., 2021; Łukasz Mikołajczak et al., 2015)

Despite the established use of *Chelidonium majus* in traditional medicine for its antimicrobial properties, most existing research has focused on alcoholic extracts, leaving a significant gap in our understanding of the biological activity of aqueous preparations. Given the increasing interest in natural, water-based remedies due to their safety, accessibility, and compatibility with various pharmaceutical formulations, it is essential to evaluate their actual efficacy. Moreover, comparative studies examining seasonal variations in plant bioactivity remain occasional. Therefore, this study aimed to investigate and compare the antimicrobial potential of both aqueous and alcoholic extracts of *Chelidonium majus*, collected during different seasons, against common bacterial and fungal pathogens. The results contribute to a better understanding of the plant's therapeutic potential and guide the development of more effective, evidence-based phytotherapeutic applications.

MATERIALS AND METHODS

Reagents and culture media. All reagents were bought from Carl Roth or Merck, Germany. Liquid Luria-Bertani (LB) medium was prepared by dissolving 5 g of yeast extract, 10 g of peptone and 10 g of NaCl in 700 mL of distilled water. pH was adjusted to 7.4 using 0.1 M sodium hydroxide and a Mettler Toledo pH meter. The final volume was made up to 1 L with distilled water, and the medium was autoclaved at 121°C for 30 minutes.

Solid LB medium was prepared in the same way as the liquid medium, with the addition of 20 g of agar before adjusting the final volume with distilled water. After autoclaving at 121°C for 30 min, the medium was poured into sterile Petri dishes.

Liquid Yeast Extract-Peptone-Dextrose (YPD) medium was prepared by dissolving 10 g of yeast extract, 20 g of peptone, and 0.08 g of adenine hemisulfate in 500 mL of distilled water under continuous stirring on a magnetic stir plate. The mixture was brought to 900 mL with additional distilled water and autoclaved at 121°C for 30 minutes. After sterilization, 100 mL of sterile 20% glucose solution was added. Solid YPD medium was prepared in the same way as the liquid medium, with the addition of 20 g of agar before autoclaving. After sterilization, 100 mL of sterile 20% glucose solution was added to complete the volume to 1 L, without any additional distilled water. The medium was then poured into sterile Petri dishes.

Microorganisms. The microorganism used (bacteria: *Escherichia coli*, *Enterobacter cloacae*, *Staphylococcus aureus*, yeast: *Candida albicans*) were purchased from ATCC, Virginia, USA.

Collecting plant material. Plant material used in the experimental procedures was collected starting in the summer of 2023. Seasonal sampling was carried out from two distinct locations: Timisoara city and Bacia commune in Hunedoara County. In addition, an extra fresh sample was collected separately from Timisoara.

Microorganisms precultures. Each 5 µL portion of stock suspension was inoculated into 5 mL of growth medium: LB broth for *Escherichia coli*, *Enterobacter cloacae*, and *Staphylococcus aureus*, and YPD broth for *Candida albicans*. Bacterial samples were incubated at 37°C for 24 hours with shaking at 250 rpm, while yeast samples were incubated at 30°C for 48 hours under the same shaking conditions.

Preparation of aqueous and alcoholic extracts of *Chelidonium majus*. To obtain *C. majus* extracts, plant material (leaves, stems, and roots) was weighed using a precision balance, with 50 g allocated for each sample. For aqueous extraction, each 50 g portion (a total of 9 samples collected across different seasons and locations) was homogenized with 50 mL of distilled water using a blender for 5 minutes. The resulting homogenates were transferred into 50 mL Falcon tubes and centrifuged at 6000 rpm for 10 minutes. Supernatants were filtered through cotton wool using a funnel, then subjected to a second centrifugation at the same speed for 15 minutes. The clarified supernatants were finally sterilized by filtration through 0.22 µm polyvinylidene fluoride (PVDF) membranes. Additionally, for fresh plant samples collected from Timisoara, alcoholic extracts were prepared under the same conditions, using 50 g of plant material homogenized with 50 mL of 50% ethanol.

Antimicrobial activity of *Chelidonium majus* aqueous and alcoholic extracts. To assess the antimicrobial activity of the *C. majus* extracts, 96-well microtiter plates were used. Each well was filled with 50 µL of LB medium (for bacterial strains) or YPD medium (for *Candida albicans*), and the extracts were diluted in sterile distilled water to obtain the following combinations (µL extract / µL sterile water): 0/50 (negative control), 1/49, 5/45, 10/40, 15/35, 20/30, 25/25, 30/20, 35/15, 40/10, 45/5, and 50/0 (undiluted extract). Additionally, extra plates were prepared to evaluate the antimicrobial effect of alcoholic extracts obtained from fresh *C. majus* material collected in Timisoara. In this case, the same dilution scheme was applied, using 50% ethanol as the negative control. Subsequently, 2 µL of precultured microbial suspensions (*Escherichia coli*, *Enterobacter cloacae*, *Staphylococcus aureus*, or *Candida albicans*) were added to each well. Initial optical density (OD_{600}) was measured immediately after inoculation (0 h), following 1 minute shaking, using a BioTek Synergy H1 microplate reader. The plates were sealed with Parafilm and incubated at 30°C with orbital shaking at 500 rpm for 24 hours. After incubation, OD_{600} readings were repeated to evaluate microbial growth inhibition in response to the extracts.

Viability assay of microorganisms treated with the alcoholic extract of *Chelidonium majus.* For the alcoholic extract and ethanol control, viability tests were made on the microbial strains. A volume of 1 µL from cultures previously exposed to either 12.5% *C. majus* alcoholic extract or 12.5% ethanol was inoculated onto Petri dishes containing LB medium (for bacteria) or YPD medium (for yeast). Each inoculum was supplemented with 100 µL of sterile distilled water. The plates were incubated overnight at 30°C and subsequently subjected to qualitative analysis and photographic documentation.

RESULTS AND DISCUSSIONS

Effect of *Chelidonium majus* aqueous extracts on strains of microorganisms

In this study, the antibacterial and antifungal effects of aqueous extracts of *C. majus* on strains of pathogenic microorganisms, *Escherichia coli*, *Staphylococcus aureus*, *Enterobacter cloacae*, and *Candida albicans* were investigated. Tests were performed using 96-well microtiter plates and the optical density of the cultures was measured at 0 h and 24 h at 600 nm. Initial measurements (0 h), showed that the initial optical density of all cultures was uniform, indicating a consistent distribution of cells in the culture medium. Measurements at 24 h, showed a significant increase in cell optical density in all wells treated with aqueous extracts of *C. majus*, suggesting a stimulation of microorganism growth rather than inhibition.

The potential impact of the harvesting location was evaluated by comparing *C. majus* plants collected from Timisoara, a more urbanized and potentially more polluted environment, with those harvested from Bacia, a rural area presumed to have lower pollution levels. The results were comparable between both sources, indicating that the antimicrobial activity of the aqueous extracts is not significantly affected by the environmental conditions of the collection site. Furthermore, a freshly prepared aqueous extract exhibited similar stimulatory effects on microbial growth, suggesting that extract freshness does not alter the biological activity. The results related to the activity of the fresh aqueous extract of *C. majus*, collected from Timisoara, are illustrated in Figure 1. To assess the effect of aqueous *C. majus* extracts on microbial growth, antimicrobial activity tests were performed. The X-axis of the resulting graphs represents the dilution levels of the plant extracts, while the Y-axis indicates the growth rate, expressed as a percentage. Growth rates were calculated based on the optical density at 600 nm of untreated control cultures, which were considered 100%. Dilutions of the extracts were performed using sterile distilled water and microorganism-specific culture media. The purpose of evaluating aqueous extracts was to investigate their potential antimicrobial properties for future use in the development of topical ointments for wound healing, free of synthetic chemical agents. The results corresponding to the aqueous extract of *C. majus*, freshly harvested in Timisoara on April 26, 2024 (Figure 1), indicates that the extract did not exhibit inhibitory activity against the tested microorganisms. On the contrary, a stimulatory effect on bacterial growth was observed for *Escherichia coli*, *Enterobacter cloacae*, and *Staphylococcus aureus*. The growth of *Candida albicans* remained comparable to that of the untreated control, showing no significant change.

Figure 2 illustrates the effects of aqueous extracts of *C. majus* collected in the summer season from Bacia (June 5, 2023) and Timisoara (June 28, 2023) on various microbial strains. The results indicate a consistent stimulatory effect on the growth of all tested microorganisms, including *Escherichia coli*, *Enterobacter cloacae*, *Staphylococcus aureus*, and *Candida albicans*. A steady increase in the growth rate was observed with increasing extract concentrations, suggesting enhanced microbial proliferation in the presence of the plant extracts. Comparative analysis of aqueous extracts of *C. majus* obtained from plants collected in Bacia and Timisoara (June 2023) revealed a consistent stimulatory effect on microbial growth, especially bacterial strains. This stimulatory response was dose-dependent, with the most concentrated conditions (lowest fold dilutions) showing the highest growth rates. In both extracts, growth of *Escherichia coli*, *Enterobacter cloacae* and *Staphylococcus aureus* increased significantly compared to the negative control, suggesting that aqueous extracts, despite

their expected antimicrobial potential, contain nutrient-like compounds and low concentrations of active phytocompounds, insufficient to inhibit bacterial proliferation under the conditions tested.

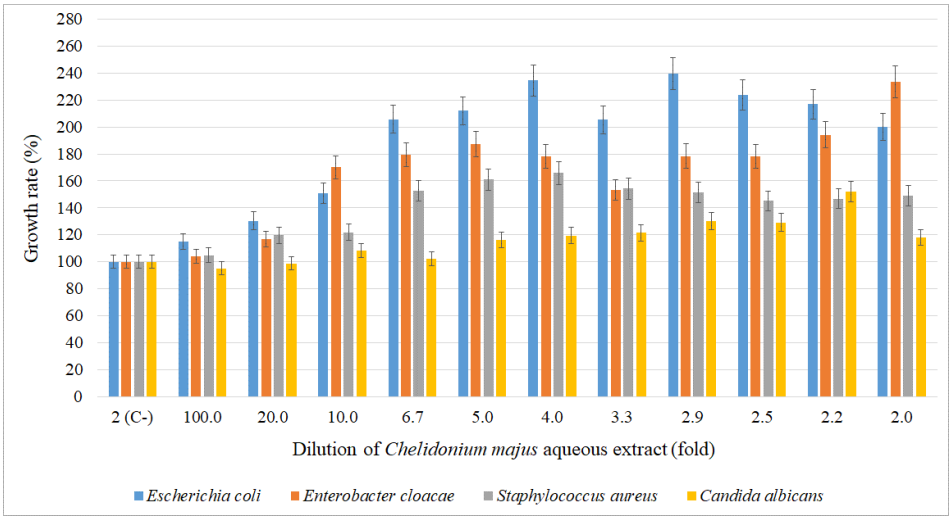

FIG 1. Effect of *Chelidonium majus* aqueous extract (freshly harvested from Timisoara) on the growth rate of microbial strains. The extract was tested at different dilutions as indicated on the X-axis. The negative control (2 (C-)) does not contain plant extract; instead, the same volume of sterile distilled water was added as for the tested samples. Thus, the culture medium in the control was diluted 1:1 (2-fold) with sterile distilled water, corresponding to the dilution level of the most concentrated extract condition. Microbial strains tested include *Escherichia coli*, *Enterobacter cloacae*, *Staphylococcus aureus* and *Candida albicans*. Results are presented as mean growth rate (%) ± standard deviation.

The extract obtained from plants collected in Timisoara (urban area) produced a slightly more pronounced stimulatory effect, especially at lower dilutions (higher extract concentration), possibly due to differences in secondary metabolite content induced by environmental stress (pollution-related oxidative stress). In contrast, no inhibitory effect against *Candida albicans* was observed in any case, with fungal growth remaining largely unchanged or slightly stimulated in all dilutions tested. This suggests that aqueous extracts do not have antifungal activity against *C. albicans*.

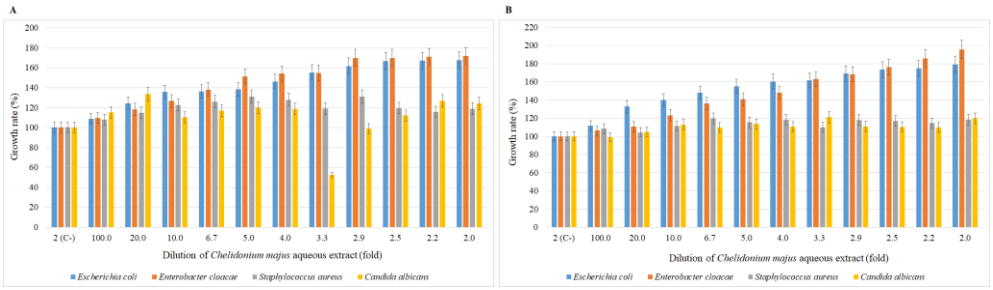

FIG 2. Effect of aqueous extracts of *Chelidonium majus* obtained from plants collected at two different locations with distinct pollution levels during the summer season. (A) Extract obtained from plants collected in Bacia (rural area with low pollution, 05.06.2023). (B) Extract obtained from plants collected in Timisoara (urban area with high pollution, 28.06.2023). Extracts were applied at different dilutions as indicated on the X-axis. The negative control (2 (C-)) did not contain any plant extract; instead, the culture medium was diluted 1:1 with sterile distilled water to correspond to the maximum dilution used in the treated samples. Microbial strains tested included *Escherichia coli*, *Enterobacter cloacae*, *Staphylococcus aureus* and *Candida albicans*. Results are expressed as mean growth rate (%) ± standard deviation.

The effects of aqueous extracts of *C. majus* harvested during the autumn season (Bacia – September 29, 2023; Timisoara – October 2, 2023) on *Escherichia coli*, *Enterobacter cloacae*, *Staphylococcus aureus*, and *Candida albicans* is presented in Figure 3. A general trend of increased microbial proliferation was observed with rising extract concentrations, indicating a concentration-dependent stimulatory effect across all tested strains. The results obtained for the extracts collected in September–October 2023 indicate that the sample from Bacia exhibited a more pronounced stimulatory effect on *Candida albicans* whereas the extract from Timisoara showed higher stimulation in the cases of *Escherichia coli* and *Enterobacter cloacae*.

Figure 4 illustrates the effects of aqueous extracts of *C. majus* harvested during the winter season (Bacia – December 21, 2023; Timisoara – December 20, 2023) on the growth of *Escherichia coli*, *Enterobacter cloacae*, *Staphylococcus aureus*, and *Candida albicans*. Comparative analysis of the aqueous extracts made from plants harvested in December 2023 revealed only minor differences in their stimulatory effects on microbial growth, between Bacia and Timisoara locations. The growth rate increased at both locations, Timisoara and Bacia, rising from approximately 100% to about 200% (samples from Bacia) and 220% (samples from Timisoara), with similar patterns observed in both locations.

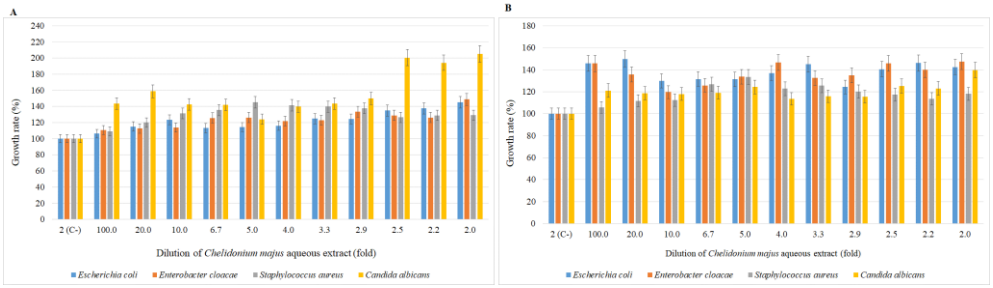

FIG 3. Effect of aqueous extracts of *Chelidonium majus* obtained from plants collected at two different locations with distinct pollution levels during the autumn season. (A) Extract obtained from plants collected in Bacia (rural area with low pollution, 29.09.2023). (B) Extract obtained from plants collected in Timisoara (urban area with high pollution, 02.10.2023). Extracts were applied at different dilutions as indicated on the X-axis. The negative control (2 (C-)) did not contain any plant extract; instead, the culture medium was diluted 1:1 with sterile distilled water to correspond to the maximum dilution used in the treated samples. Microbial strains tested included *Escherichia coli*, *Enterobacter cloacae*, *Staphylococcus aureus* and *Candida albicans*. Results are expressed as mean growth rate (%) ± standard deviation.

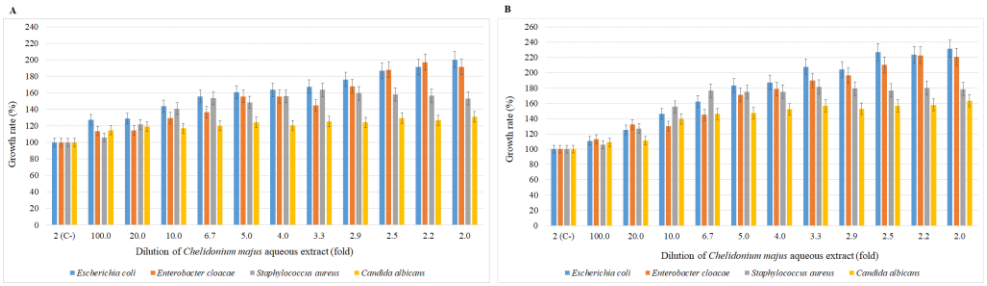

FIG 4. Effect of aqueous extracts of *Chelidonium majus* obtained from plants collected at two different locations with distinct pollution levels during the winter season. (A) Extract obtained from plants collected in Bacia (rural area with low pollution, 21.12.2023). (B) Extract obtained from plants collected in Timisoara (urban area with high pollution, 20.12.2023). Extracts were applied at different dilutions as indicated on the X-axis. The negative control (2 (C-)) did not contain any plant extract; instead, the culture medium was diluted 1:1 with sterile distilled water to correspond to the maximum dilution used in the treated samples. Microbial strains tested included *Escherichia coli*, *Enterobacter cloacae*, *Staphylococcus aureus* and *Candida albicans*. Results are expressed as mean growth rate (%) ± standard deviation.

Figure 5 presents the effects of aqueous extracts prepared from plants collected in the spring (Bacia – April 4, 2024; Timisoara – April 4, 2024) on the growth of *Escherichia coli*, *Enterobacter cloacae*, *Staphylococcus aureus*, and *Candida albicans*. An increase in extract volume corresponded to an overall stimulation of microbial growth across all tested strains.

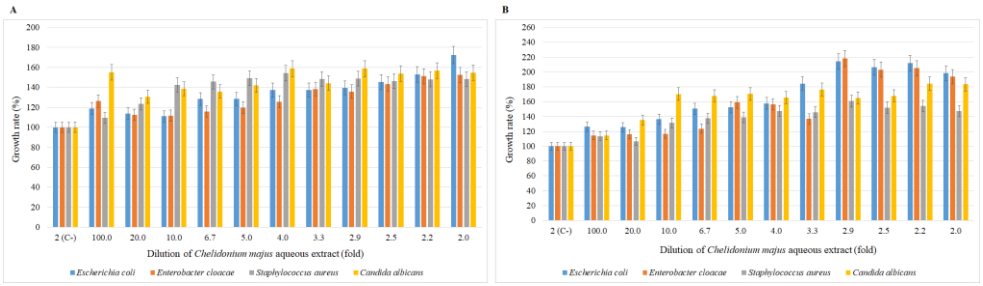

FIG 5. Effect of aqueous extracts of *Chelidonium majus* obtained from plants collected at two different locations with distinct pollution levels during the spring season. (A) Extract obtained from plants collected in Bacia (rural area with low pollution, 04.04.2024). (B) Extract obtained from plants collected in Timisoara (urban area with high pollution, 04.04.2024). Extracts were applied at different dilutions as indicated on the X-axis. The negative control (2 (C-)) did not contain any plant extract; instead, the culture medium was diluted 1:1 with sterile distilled water to correspond to the maximum dilution used in the treated samples. Microbial strains tested included *Escherichia coli*, *Enterobacter cloacae*, *Staphylococcus aureus* and *Candida albicans*. Results are expressed as mean growth rate (%) ± standard deviation.

The most important differences were observed between the freshly harvested extracts from Bacia and Timisoara on the same date. The Bacia extract exhibited a moderate stimulatory effect similar to previous samples, particularly on bacterial strains (the growth rate was over 120% for all strains). In contrast, the extract from Timisoara induced an increase in *Candida albicans* proliferation, suggesting a potential influence of local environmental factors on the plant's chemical composition. One plausible explanation is that the plant collected in Timisoara may have absorbed higher levels of soil minerals known to enhance the growth of *Candida albicans*, such as aluminum (Al), manganese (Mn), and sodium (Na), as reported in previous studies (Sautour et al., 2021). It is well established that, during spring, plants intensify nutrient uptake from the soil to support rapid growth and flowering. This seasonal physiological behaviour may account for the variations in extract composition and the differential effects observed on microbial strains.

Effect of alcoholic extracts of *Chelidonium majus* on microorganism strains

Scientific literature has consistently reported the antimicrobial properties of *C. majus* alcoholic extracts. In the present study, the antimicrobial activity of an ethanolic extract of *C. majus* was evaluated and compared with that of the corresponding aqueous extract and pure ethanol. Ethanol (Figure 6) and the ethanolic extract (Figure 7) were tested across a concentration range of 0% to 25%. At a concentration of 12.5% ethanol, microbial growth was almost completely inhibited for all tested strains.

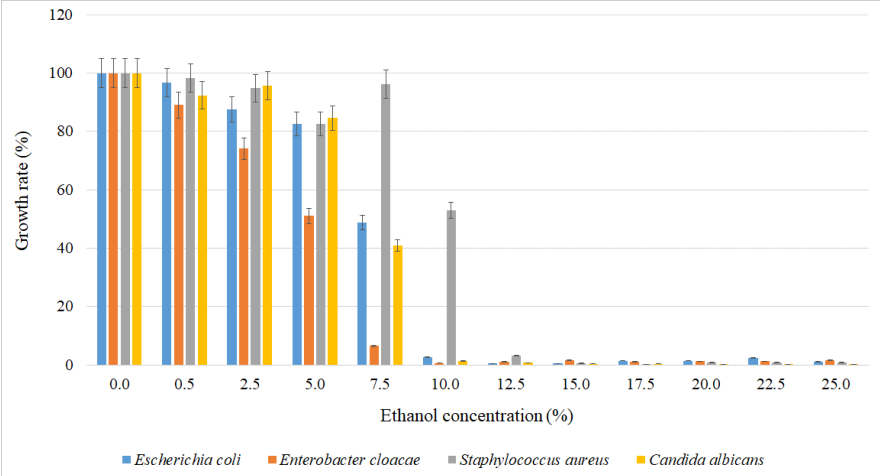

FIG 6. Antimicrobial effect of ethanol on various microbial strains tested at concentrations ranging from 0% to 25%. Microbial strains tested included *Escherichia coli*, *Enterobacter cloacae*, *Staphylococcus aureus* and *Candida albicans*.

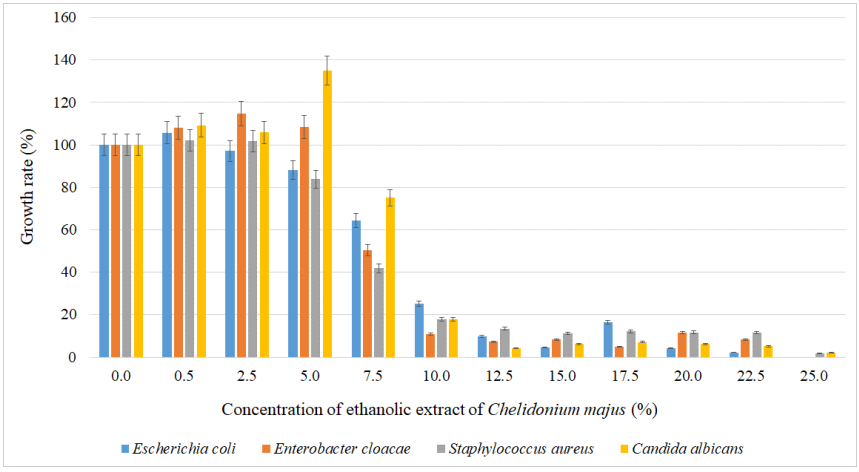

FIG 7. Antimicrobial effect of alcoholic extract of freshly collected *Chelidonium majus* from Timisoara on various microbial strains tested at concentrations ranging from 0% to 25%. Microbial strains tested included *Escherichia coli*, *Enterobacter cloacae*, *Staphylococcus aureus* and *Candida albicans*. Results are expressed as mean growth rate (%) ± standard deviation.

However, at lower concentrations (≤5%), neither pure ethanol nor the ethanolic extract exhibited significant antimicrobial activity. *C. albicans* showed a obvious increase in growth at certain ethanolic extract concentrations, with a peak proliferation observed at 5% ethanol. This finding suggests that *C. albicans* can tolerate moderate concentrations of ethanol, and at 5%, ethanol can induce a mild stress that stimulates certain metabolic processes or inhibits competitors, resulting in apparently accelerated growth.

Viability test of microorganism strains exposed to ethanol and *Chelidonium majus* alcoholic extract

The results obtained from optical density measurements of *Chelidonium majus* alcoholic extracts were corroborated by microbial viability assays. These tests confirmed that the alcoholic extract exerted both bacteriostatic and fungistatic effects (Figure 8). The most pronounced antimicrobial activity of ethanol was observed against *Staphylococcus aureus* and *Candida albicans*, where the effects appeared to be slightly bactericidal and fungicidal, respectively, as evidenced by a reduced number of colonies per 1 µL of treated culture. This observation is in accordance with existing literature (Colombo & Bosisio, 1996), which highlights the broad-spectrum antiviral, antitumor, and antimicrobial effects of *C. majus* extracts and isolated compounds in both *in vitro* and *in vivo* models. Furthermore, the viability test performed on microbial strains exposed to 12.5% ethanol (Figure 9) revealed comparable results to those obtained with the alcoholic extract of *C. majus*, suggesting that ethanol is the primary contributor to the observed antimicrobial activity.

In addition to its antimicrobial effects, the extract of *C. majus* has been shown to be safe for use in veterinary and human phytopreparations. In various complementary and alternative medicine systems, including homeopathy, different parts of this plant are used to treat gastric ulcers, gastric cancer, oral infections, liver diseases, general pain, and various skin conditions. Extracts from the leaves, flowers, and roots are used internally to stimulate bile production and pancreatic digestive enzymes. Due to its choleretic and spasmolytic properties, *C. majus* is widely used for the treatment of biliary disorders, dyspepsia, and irritable bowel syndrome. In homeopathic medicine, highly diluted extracts of *C. majus* are used against various forms of liver diseases, including liver cancer. Phytochemical analysis has revealed the presence of numerous active compounds such as chelidonine, chelerythrine, sanguinarine, berberine, protopine, allocryptopine, coptisine, and others. Both the crude extract and purified constituents of *C. majus* have shown a wide range of pharmacological activities, including anti-

inflammatory, antimicrobial, immunomodulatory, anticancer, hepatoprotective, and analgesic effects (Amal & Pratim, 2015).

The therapeutic effect of *Chelidonium majus* latex against the human papillomavirus (HPV) is well known; however, the molecular mechanism behind this activity is not yet fully understood. The latex of *Chelidonium majus* contains a variety of chemically active compounds, such as proteins and alkaloids, which may inhibit the replication of HPV by acting on different stages of its life cycle. The antiviral and antitumor properties observed in *C. majus* latex are often attributed to the alkaloids it contains; however, recent studies suggest that proteins in the latex may also play a significant role in its biological and pharmacological activities (Musidlak et al., 2022).

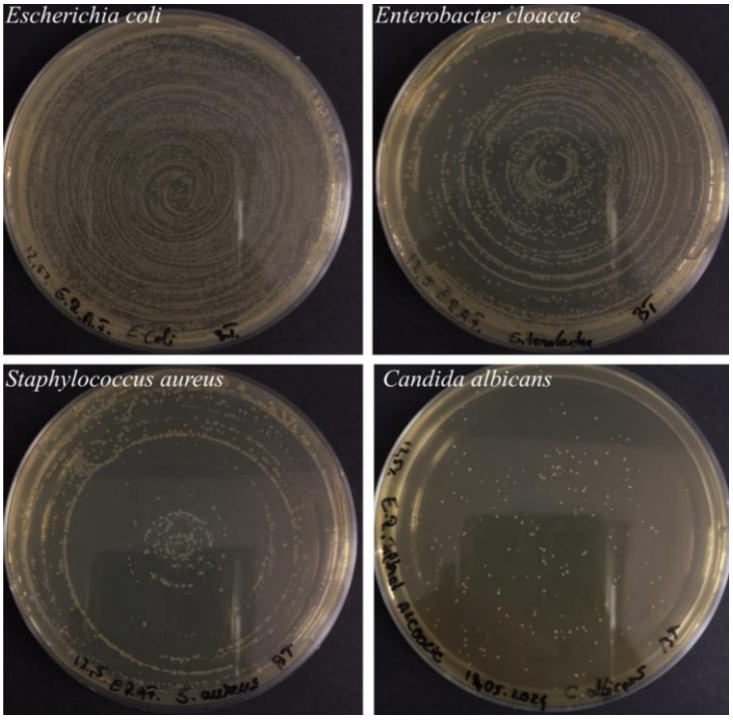

FIG 8. Viability test of microorganism strains exposed to the ethanolic extract of *Chelidonium majus* at a 12.5% ethanol concentration. A volume of 1 µL from each microbial strain (*Escherichia coli*, *Enterobacter cloacae*, *Staphylococcus aureus* and *Candida albicans*), previously exposed for 24 hours to different ethanolic plant extract concentrations, was plated for viability assessment.

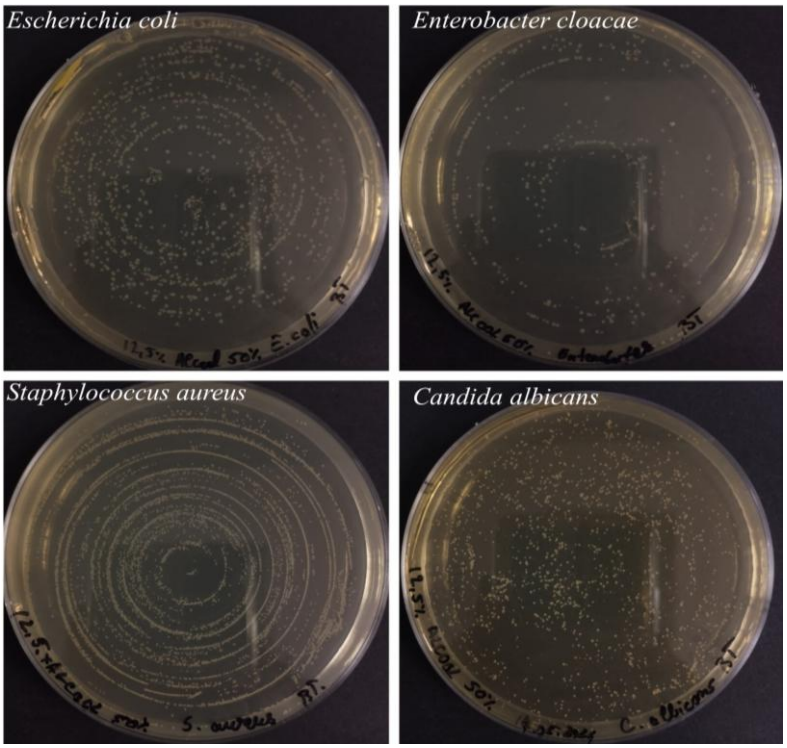

FIG 9. Viability test of microorganism strains exposed to the ethanol at a 12.5% concentration. A volume of 1 µL from each microbial strain (*Escherichia coli*, *Enterobacter cloacae*, *Staphylococcus aureus* and *Candida albicans*), previously exposed for 24 hours to different ethanol concentrations, was plated for viability assessment.

CONCLUSIONS

In this study, alcoholic extracts of *Chelidonium majus* were shown to exert a significant inhibitory effect on the growth of microorganisms such as *Escherichia coli*, *Enterobacter cloacae*, *Staphylococcus aureus* and *Candida albicans*. In contrast, the aqueous extracts showed no antimicrobial or antifungal activity. The exposure to aqueous extracts resulted in an increase in microbial growth, confirming that water is not an effective solvent for the extraction of bioactive antimicrobial compounds from *Chelidonium majus*. However, when comparing the antimicrobial effect of the alcoholic plant extract with that of ethanol alone, no substantial differences in microbial growth inhibition were observed. These results confirm that the antimicrobial activity is

primarily attributed to the presence of ethanol rather than to the plant-derived compounds in the alcoholic extract.

Consequently, the extraction method is of crucial importance in determining the biological efficacy of herbal-derived preparations. The choice of solvent significantly influences the recoverability of the active compounds and should therefore be carefully considered when aiming to obtain extracts with antimicrobial potential from *Chelidonium majus*.

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