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CHEMICAL CHARACTERIZATION AND DETERGENT POTENTIAL OF *CHELIDONIUM MAJUS* EXTRACTS

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ABSTRACT. The present study investigates the impact of different extraction methods—accelerated solvent extraction (ASE), ultrasound-assisted extraction (UAE), and maceration on the yield, phytochemical composition, antioxidant activity, and antimicrobial potential of *Chelidonium majus* extracts. ASE demonstrated the highest extraction yield (85.7%) and alkaloid content (5.4 mg AE/ml), correlating with enhanced antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans* (lowest MIC values: 3.12%, 2.50%, and 6.25%, respectively). UAE resulted in the highest polyphenol content (15.2 mg GAE/ml) and antioxidant capacity (12.8 ± 1.5 µmol Trolox/ml), suggesting its effectiveness in preserving antioxidant compounds. Maceration produced the lowest bioactive compound yield and biological activity. These findings indicate that ASE is optimal for antimicrobial applications, while UAE is preferable for antioxidant-enriched extracts. The results provide valuable insights for optimizing *C. majus* extraction in pharmaceutical, food, and cosmetic formulations.

Keywords: *Chelidonium majus, accelerated solvent extraction, ultrasound-assisted extraction, alkaloids, flavonoids, polyphenols, antimicrobial activity, antioxidant capacity, extraction efficiency*

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INTRODUCTION

Chelidonium majus L., commonly known as Greater Celandine, is a perennial herbaceous plant belonging to the Papaveraceae family. It has been traditionally used in herbal medicine due to its diverse pharmacological properties, including antimicrobial, anti-inflammatory, antioxidant, and antifungal activities. The plant is widely distributed across Europe and Asia, thriving in temperate climates and commonly found in meadows, forest edges, and near human settlements [1, 2].

The bioactive potential of *C. majus* is attributed to its rich phytochemical composition, predominantly consisting of alkaloids, flavonoids, saponins, and essential oils [1, 3]. The most notable compounds in *C. majus* are isoquinoline alkaloids, including chelidonine, sanguinarine, berberine, chelerythrine, and coptisine, which exhibit strong antimicrobial and antifungal effects [4, 5]. These alkaloids are responsible for the plant's characteristic yellow-orange latex, which has been historically used for its therapeutic benefits [6, 7].

In addition to alkaloids, *C. majus* contains significant amounts of flavonoids and polyphenolic compounds, which contribute to its antioxidant properties [1, 8, 9]. These bioactive molecules scavenge free radicals and provide stability to formulations that are prone to oxidative degradation. The presence of saponins further enhances the plant’s detergent potential by acting as natural surfactants with foaming and emulsifying properties [10].

Given its unique chemical profile, *C. majus* presents a promising alternative to synthetic detergent additives. Its natural surfactant-like components, antimicrobial properties, and antioxidant capacity make it a viable candidate for eco-friendly detergent formulations [11]. However, a thorough chemical characterization and functional assessment are necessary to evaluate its suitability in detergent applications.

The growing demand for eco-friendly and sustainable cleaning products has led to increased research on plant-derived detergent ingredients [12,13]. Various plant-based compounds, such as saponins, flavonoids, and essential oils, have been explored for their surfactant, antimicrobial, and antioxidant properties, making them viable alternatives to synthetic detergent components [14]. Saponin-rich plants, such as *Sapindus mukorossi* (soapnut), *Quillaja saponaria* (soapbark tree), and *Glycyrrhiza glabra* (licorice), have been extensively studied for their natural foaming and emulsifying abilities, demonstrating their effectiveness in household and personal care formulations [15-18]. Studies have also highlighted the antimicrobial potential of plant extracts, particularly those containing alkaloids, tannins, and phenolics, which can enhance the hygienic properties of detergents by inhibiting bacterial and fungal growth [19, 20].

Several investigations have focused on the integration of essential oils from plants like *Eucalyptus globulus*, *Cymbopogon citratus* (lemongrass), and *Thymus vulgaris* (thyme) due to their broad-spectrum antimicrobial activity and pleasant aromatic properties [21, 22]. These essential oils have shown promising results in inhibiting common detergent contaminants such as *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*, thus contributing to the formulation of natural disinfectant detergents [23, 24]. Additionally, polyphenolic-rich extracts from plants like *Camellia sinensis* (green tea) and *Punica granatum* (pomegranate) have been investigated for their antioxidant stability, preventing oxidative degradation of detergent formulations and enhancing product shelf life [25, 26].

While extensive research has been conducted on these plant-based surfactants and antimicrobial agents, fewer studies have explored the potential of alkaloid-containing plants such as *C. majus* for detergent applications. Given its rich composition of isoquinoline alkaloids, saponins, and flavonoids, *C. majus* represents a novel and underexplored candidate for natural detergent formulations. This study seeks to fill this research gap by evaluating its chemical composition, surfactant properties, and antimicrobial potential in detergent applications.

RESULTS AND DISCUSSION

Extraction Yield and Phytochemical Composition

The extraction efficiency and phytochemical composition of *C. majus* extracts varied depending on the method employed (Table 1). Accelerated Solvent Extraction (ASE) demonstrated the highest extraction yield (85.7%), significantly surpassing Ultrasound-Assisted Extraction (UAE) (72.3%) and Maceration (68.5%). This trend indicates that high pressure and controlled temperature in ASE enhance solvent penetration, leading to a more efficient recovery of bioactive compounds.

ASE produced the highest alkaloid concentration (5.4 mg AE/ml), followed by UAE (4.1 mg AE/ml) and maceration (3.2 mg AE/ml). Given that alkaloids such as chelidonine, sanguinarine, and chelerythrine contribute to antimicrobial activity, the higher yield in ASE correlates with the stronger antimicrobial properties observed in MIC testing. The total flavonoid concentration was highest in ASE (9.3 mg QE/ml), indicating that high-pressure extraction preserves and extracts a broader range of flavonoids. UAE was slightly lower (8.8 mg QE/ml), while maceration resulted in the lowest yield (8.5 mg QE/ml). Flavonoids are potent antioxidants, making these results relevant for applications

in detergent formulations requiring oxidative stability. UAE extracts had the highest total polyphenol content (15.2 mg GAE/ml), followed by ASE (14.1 mg GAE/ml) and maceration (12.5 mg GAE/ml). The superior performance of UAE in polyphenol recovery is attributed to ultrasound-induced cell wall disruption, allowing a more efficient release of these bioactive compounds. The pH of all extracts remained in a narrow range (5.5–5.8), which is considered suitable for detergent formulations. Maintaining a balanced pH is essential to ensure product stability and compatibility with surfactants, as extreme pH variations could affect the performance and shelf-life of the formulation (Figure 1).

Table 1. Extraction Yield and Phytochemical Composition of *C. majus* Extracts

Extraction Method	Polyphenol Yield (%)	Total Alkaloids (mg AE/ml)	Total Flavonoids (mg QE/ ml)	Total Polyphenols (mg GAE/ ml)	pH
Maceration (5 h, 22–25°C)	68.5 ± 10.2	3.2 ± 0.4	8.5 ± 0.8	12.5 ± 1.0	5.8 ± 0.2
UAE (1 h, 45°C)	72.3 ± 8.5	4.1 ± 0.5	8.8 ± 0.9	15.2 ± 1.2	5.6 ± 0.1
ASE (35°C, 10.3 MPa)	85.7 ± 9.8	5.4 ± 0.6	9.3 ± 0.7	14.1 ± 1.3	5.5 ± 0.1

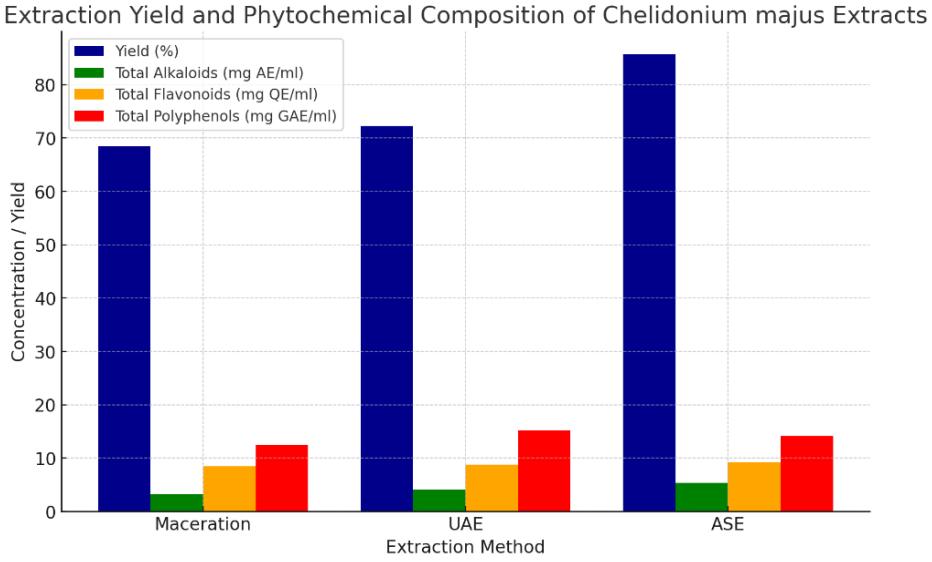

Figure 1. Extraction yield and phytochemical composition of *C. majus* extracts

The higher yield and alkaloid content in ASE extracts suggest that high-pressure conditions enhance the extraction of bioactive compounds with antimicrobial properties, supporting their application in eco-friendly detergent formulations. Meanwhile, UAE appears to be the most effective in polyphenol extraction, making it a preferable method for producing antioxidant-rich extracts.

In comparison, a study by Boia et al. identified 74 phytochemicals in Romanian wild-grown *C. majus*, including alkaloids, amino acids, phenolic acids, flavonoids, carotenoids, fatty acids, sterols, and others. However, this study did not specify extraction yields or compare different extraction methods [2].

Another study evaluated various extraction techniques—aqueous and alcoholic extraction, supercritical fluid extraction, pressing-centrifugation, and microwave extraction—but did not provide specific yields or phytochemical concentrations for comparison [27].

Antimicrobial Activity (MIC Values)

The antimicrobial potential of *C. majus* extracts obtained through maceration, ultrasonic-assisted extraction (UAE), and accelerated solvent extraction (ASE) was evaluated against *Escherichia coli* (Gram-negative), *Staphylococcus aureus* (Gram-positive), and *Candida albicans* (fungal species). The results of the minimum inhibitory concentration (MIC) assay are summarized in Table 2. The MIC values are expressed as % (v/v), representing the volume of extract per volume of testing solution (e.g., 6.25% extract means 6.25 µL extract in 100 µL total solution).

Table 2. Minimum Inhibitory Concentration (MIC) of *C. majus* Extracts (v/v %)

Microorganism	Maceration	UAE	ASE	Positive Control
E. coli	12.5% ± 1.2	6.25% ± 0.8	3.12% ± 0.6	Ampicillin (2 µg/ml)
S. aureus	10.0% ± 1.0	5.00% ± 0.7	2.50% ± 0.5	Vancomycin (1 µg/ml)
C. albicans	25.0% ± 1.5	12.5% ± 1.0	6.25% ± 0.8	Fluconazole (1 µg/ml)

The data demonstrate that the antimicrobial efficacy of *C. majus* extracts was strongly influenced by the extraction method used. ASE consistently exhibited the lowest MIC values across all tested microorganisms, indicating the highest antimicrobial potency, followed by UAE, while maceration resulted in the weakest inhibitory effect (Figure 2).

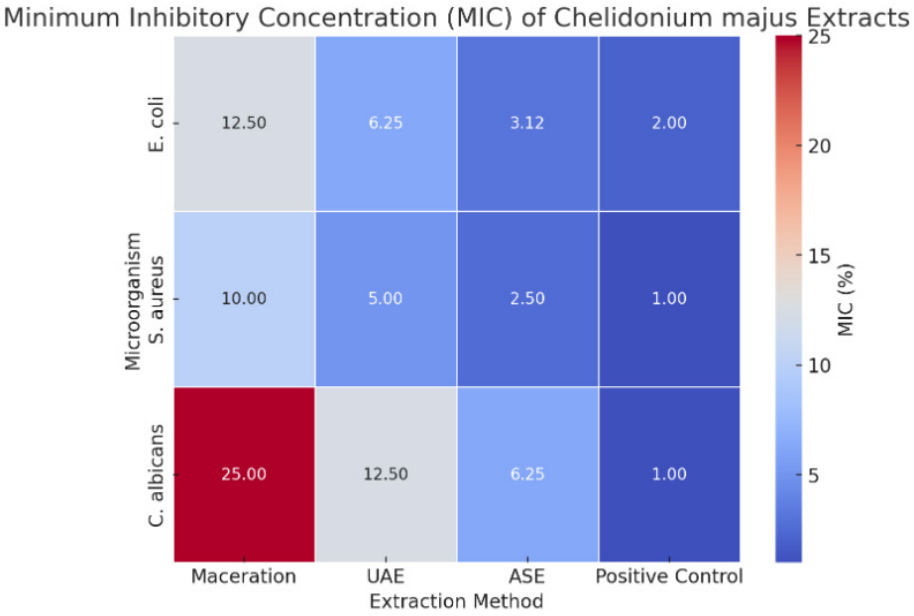

Figure 2. Minimum Inhibitory Concentration (MIC) of *C. majus* extracts

The ASE extract displayed the strongest inhibition of *E. coli* (MIC = 3.12% ± 0.6), while UAE showed moderate efficacy (MIC = 6.25% ± 0.8), and maceration exhibited the highest MIC (12.5% ± 1.2%). The Gram-negative bacterial cell wall, which contains an outer membrane rich in lipopolysaccharides, is known to limit the penetration of antimicrobial compounds, which may account for the relatively higher MIC values compared to *S. aureus*. However, ASE-extracted bioactive compounds approached the inhibitory strength of the positive control (ampicillin, 2 µg/ml), suggesting its potential as a natural antibacterial agent.

The extracts demonstrated higher inhibition against *S. aureus* compared to *E. coli*, with ASE again yielding the lowest MIC (2.50% ± 0.5%), followed by UAE (5.00% ± 0.7%) and maceration (10.0% ± 1.0%). The increased sensitivity of *S. aureus* is likely due to the lack of an outer membrane, making it more susceptible to plant-derived alkaloids and polyphenols. The MIC of ASE was relatively close to vancomycin (1 µg/ml), suggesting the extract's strong antibacterial potential against Gram-positive pathogens.

The antifungal activity of the extracts followed the same trend as antibacterial efficacy, with ASE showing the lowest MIC (6.25% ± 0.8%), UAE demonstrating moderate inhibition (12.5% ± 1.0%), and maceration being the least effective (25.0% ± 1.5%). These results indicate that higher

extract concentrations were required for fungal inhibition compared to bacteria, possibly due to the thicker fungal cell wall composed of chitin and glucans, which provides structural resistance. Despite showing promising antifungal activity, the extracts did not reach the potency of the positive control fluconazole (1 µg/ml).

One-way ANOVA followed by Tukey's post-hoc test confirmed statistically significant differences ($p < 0.05$) among extraction methods, with ASE extracts exhibiting significantly lower MIC values compared to UAE and maceration. These findings highlight the superior efficiency of ASE in extracting antimicrobial bioactives from *C. majus*.

Similarly, a study by Krzyżek et al. (2021) found that *C. majus* extracts demonstrated antimicrobial activity against Helicobacter pylori, with MIC value of 128 µg/ml for root extracts [28]. This aligns with our findings that *C. majus* extracts possess significant antimicrobial properties [1].

Antioxidant Activity

Table 3 shows the antioxidant capacity in Trolox equivalents. The antioxidant capacity of *C. majus* extracts varied significantly depending on the extraction method employed, as measured in µmol Trolox/ml extract. Among the three methods tested, ultrasound-assisted extraction (UAE) exhibited the highest antioxidant capacity (12.8 ± 1.5 µmol Trolox/ml), followed by accelerated solvent extraction (ASE) (10.9 ± 1.3 µmol Trolox/ml), while maceration resulted in the lowest antioxidant yield (8.5 ± 1.2 µmol Trolox/ml). These findings highlight the crucial role of extraction technique in maximizing the recovery of bioactive compounds with antioxidant properties.

Table 3. Antioxidant Capacity of *C. majus* Extracts

Extraction Method	Antioxidant Capacity (µmol Trolox/ml extract)
Maceration	8.5 ± 1.2
UAE	12.8 ± 1.5
ASE	10.9 ± 1.3

A study by Dumitru and Gănescu assessed the antioxidant activity of alcoholic extracts of C. majus flowers using the DPPH method and found notable antioxidant activity, though specific values were not provided [2].

CONCLUSIONS

The extraction method significantly influenced the yield, phytochemical composition, antioxidant capacity, and antimicrobial activity of *C. majus* extracts. Accelerated solvent extraction (ASE) demonstrated the highest overall yield (85.7%) and alkaloid content (5.4 mg AE/ml), correlating with its superior antimicrobial activity (lowest MIC values). Ultrasound-assisted extraction (UAE) was the most effective for polyphenol recovery (15.2 mg GAE/ml) and exhibited the highest antioxidant capacity (12.8 ± 1.5 µmol Trolox/ml).

ASE extracts showed the strongest antimicrobial activity, particularly against *E. coli, S. aureus*, and *C. albicans*, likely due to their higher alkaloid and flavonoid content. In contrast, UAE produced extracts with enhanced antioxidant properties, suggesting its suitability for applications where oxidative stability is critical. Maceration yielded the lowest bioactive compound recovery and biological activity.

Overall, ASE is optimal for antimicrobial-rich extracts, while UAE is preferable for antioxidant applications. These findings provide a basis for selecting appropriate extraction techniques depending on the intended application of *C. majus* bioactive. Future studies should explore the stability and bioavailability of these extracts in formulated products.

EXPERIMENTAL SECTION

Plant Material and Extraction

Collection and Preparation of *C. majus* Samples

The aerial parts of *C. majus* were collected from the Cluj County area during the optimal harvesting period, which typically falls between May and July when the plant reaches peak biosynthesis of bioactive compounds such as alkaloids, flavonoids, and saponins. Fresh plant material was air-dried in a well-ventilated, shaded area to prevent degradation of thermolabile compounds. The dried samples were then ground into a fine powder to facilitate efficient extraction.

Reagents

All solvents were sourced from VWR (Darmstadt, Germany), while ultra-pure water was obtained using the ULTRACLEAR UV UF EVOQUA Purification System (Pittsburgh, PA, USA). The ACL Kit was supplied by Analytik Jena (Jena, Germany). All other standards used in the analysis were purchased from Sigma-Aldrich (Saint Louis, MO, USA).

Extraction Methods

To assess the efficiency of different extraction techniques, three distinct methods were employed: maceration, ultrasonic-assisted extraction (UAE), and accelerated solvent extraction (ASE). The same extraction solvent mixture of ethanol-water (2:3, v/v) was used for all extractions to ensure consistency in solvent polarity and compound solubility. 10 g of dry powdered plant material was extracted with 100 mL of solvent in each method.

Maceration was conducted at ambient temperature (~22–25°C) for 5 hours under continuous stirring to allow passive diffusion of bioactive compounds into the solvent. The extract was then filtered and stored at 4°C until further analysis.

Ultrasonic-Assisted Extraction (UAE) was performed at 45°C for 1 hour using an ultrasonic bath at 59 kHz. The ultrasonic waves facilitated cell wall disruption, enhancing the release of phytochemicals into the extraction medium. The extract was then filtered and stored at 4°C until further analysis.

Accelerated Solvent Extraction (ASE) was carried out using a Thermo Scientific™ Dionex™ ASE 350 Accelerated Solvent Extractor at high pressure (10.3 MPa) and 35°C, ensuring rapid and efficient extraction while minimizing thermal degradation of sensitive compounds. The extracts were stored at 4°C until further analysis.

Phytochemical Composition Analysis

Total Alkaloids - The quantification of total alkaloids in *C. majus* extracts was performed using UV-Vis spectrophotometry. The alkaloid content was determined based on complex formation with a chromogenic reagent, followed by absorbance measurement at a specific wavelength. The results were expressed as milligrams of alkaloid equivalents per ml of extract (mg AE/ml). 1 ml of extract was diluted with 10 ml methanol (MeOH). The solution was then mixed with the 1 ml Dragendorff's reagen and 1,5 ml Bromocresol Green (BCG)buffer solutions and allowed to react for 30 minutes at room temperature to develop the color. The absorbance of the alkaloid-dye complex was measured at 470 nm. A standard calibration curve was constructed using berberine as reference alkaloids (5–50 µg/ml).

Total Polyphenols - The polyphenol content was determined using the Folin-Ciocalteu method, a widely used spectrophotometric assay for phenolic compounds. The reaction of polyphenols with the Folin-Ciocalteu reagent resulted in a blue complex, whose absorbance was measured at 765 nm. The total polyphenol content was expressed as mg gallic acid equivalents per ml of extract (mg GAE/ml).

Polyphenol Yield Calculation - The extraction yield for polyphenols was calculated based on the total amount of polyphenols recovered in the extract relative to the total polyphenol content measured in the raw plant material. The following formula was used:

$$Polyphenol\ Yield\ (\%) = \left(\frac{Total\ polyphenols\ in\ extract}{Total\ polyphenols\ in\ plant\ material}\right) x100$$

The total polyphenol content of the unextracted plant material was determined separately using methanol as solvent and the same Folin-Ciocalteu method.

Total Flavonoids - The total flavonoid content was assessed using UV-Vis spectrophotometry, employing a colorimetric method based on the reaction with aluminum chloride ($AlCl_3$). 1 ml of extract was diluted with 10 ml methanol (MeOH). To each extract solution, 1 ml 2% $AlCl_3$ was added and mixed thoroughly. The reaction mixture was allowed to incubate for 30 minutes at room temperature in the dark to prevent degradation. Absorbance was measured at 415 nm. The calibration curve was prepared using quercetin (standard solutions at different concentrations (5–50 µg/ml), ensuring $R^2 \geq 0.99$ for accuracy. The total flavonoids content was expressed as mg quercetin equivalents per ml of extract (mg QE/ml).

pH Measurement - The pH of the extracts was measured using a calibrated pH meter to assess their suitability for detergent formulations. Maintaining an optimal pH is crucial for ensuring the stability and performance of the detergent, as well as its compatibility with other formulation components.

Antimicrobial and Antioxidant Properties

Antimicrobial Activity – Minimum Inhibitory Concentration (MIC) Testing The antimicrobial activity of *C. majus* extracts was evaluated using the Minimum Inhibitory Concentration (MIC) assay, which determines the lowest extract concentration required to inhibit the visible growth of microbial contaminants commonly found in detergent formulations. The test was performed against Gram-negative bacteria (*Escherichia coli*), Gram-positive bacteria (*Staphylococcus aureus*), and fungal species (*Candida albicans*), which are representative of potential spoilage microorganisms and human pathogens in detergent environments. Bacterial strains used were *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 29213) and fungal strain was *Candida albicans* (ATCC 10231). The culture medium used was Mueller-Hinton

broth (MHB) for bacteria and Sabouraud dextrose broth (SDB) for *C. albicans.* Incubation was done at 37°C for 18–24 hours for bacteria and 30°C for 24–48 hours for fungi. The liquid extracts were diluted in sterile distilled water or Mueller-Hinton broth (for bacteria) and Sabouraud broth (for fungi) to prepare serial two-fold dilutions ranging from 100% (undiluted) to 0.1% (v/v) to determine the lowest effective concentration. Controls included: negative control (growth control) were microorganisms in broth without the extract to ensure normal growth; positive control: were standard antibiotics (ampicillin for *E. coli*, vancomycin for *S. aureus*, and fluconazole for *C. albicans*) and solvent control: control wells containing only the extraction solvent (ethanol-water 2:3, v/v) were included to verify the solvent did not inhibit microbial growth. The MIC was determined using the broth microdilution method in a 96-well microplate, a standard method for assessing antimicrobial activity of liquid samples.

The microbial inoculum was prepared from fresh bacterial cultures that were standardized to 0.5 McFarland standard (~1.5×10^8 CFU/ml) using a spectrophotometer at 600 nm. The suspension was diluted 1:100 in broth to reach a final concentration of 5×10^5 CFU/ml per well. 100 µL of liquid extract at the highest concentration (100% v/v) was added to the first row of wells. Two-fold serial dilutions were performed by transferring 100 µL to the next well, ensuring concentrations from 100% down to 0.1% (v/v) were tested. 100 µL of standardized microbial suspension was added to each well, resulting in a final volume of 200 µL per well. The plate was incubated at 37°C for 18–24 hours for bacteria and 30°C for 24–48 hours for fungi under aerobic conditions. The MIC was recorded as the lowest concentration of the extract where no visible microbial growth (turbidity) was observed. Optical density (OD) was measured at 600 nm using a microplate reader to confirm microbial inhibition. A 0.015% resazurin solution was added to the wells and incubated for an additional 2 hours to confirm viability (live cells reduce resazurin to a pink color, while inhibited cells remain blue). The MIC was expressed as the lowest concentration of *C. majus* extract (v/v) that completely inhibited microbial growth. The antimicrobial effectiveness was compared across different extraction methods (maceration, UAE, ASE) to determine the most potent extract.

Antioxidant capacity - 1 ml of extract was diluted with 10 ml MeOH and then directly injected in to PHOTOCHEM, Analytik Jena, Germany and the antioxidant capacity was measured suing the ACL kit and expressed in equivalent Trolox. The samples were done in triplicate.

Statistical evaluation - The results were expressed as mean ± standard deviation, and statistical analysis was performed using one-way analysis of variance (ANOVA) in Minitab for Windows, version 17.0 (Minitab, LLC, State College, PA, USA).

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