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Microwave-assisted vs. conventional hydrodistillation of *Corchorus olitorius*: a chemical and enzyme inhibition study

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Author contributions

All authors contributed to the manuscript. Sameh S carried out the experiments, interpreted the results, and wrote the original draft; Elissawy AM contributed by performing formal analysis, validation, writing-review, editing and supervision; Al-Sayed E, Labib RM, Chang FR contributed by performing formal analysis, methodology, validation, writing-review, editing and supervision; and Singab ANB was responsible for conceptualization, supervision of the work, revising the interpreted results, reviewing and editing the manuscript, and giving final approval for the version to be published.

Competing interests

The authors declare no conflicts of interest.

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Abbreviations

MAHD, microwave-assisted hydrodistillation; HD, hydrodistillation; GC/MS, gas chromatography/mass spectrometry; RI, retention index.

Citation

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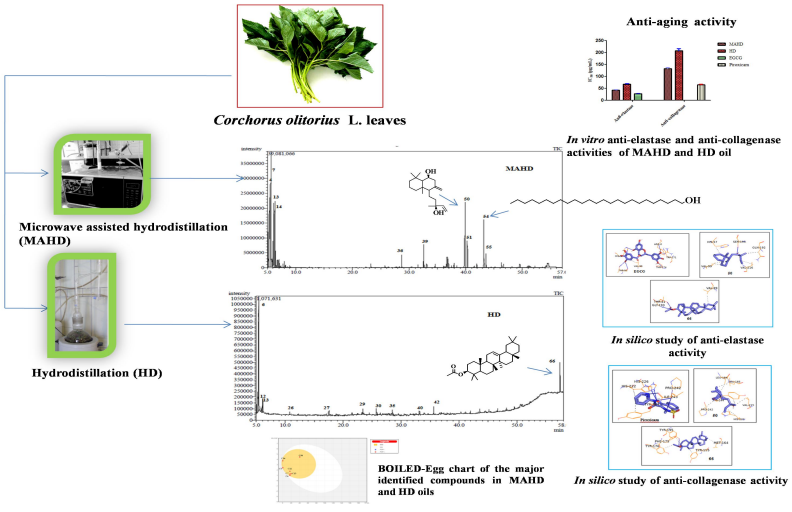
Abstract

Background: *Corchorus olitorius* L. (Malvaceae) is a green leafy vegetable widely consumed in the Middle East and valued for its rich nutritional content. Its essential oils, an important class of secondary metabolites, are of growing interest for potential use in cosmetics and fragrance industries due to their bioactive properties. **Methods**: Leaves of *C. olitorius* cultivated in Egypt were extracted using microwave-assisted hydrodistillation (MAHD) and conventional hydrodistillation (HD). The oils were analyzed by gas chromatography-mass spectrometry (GC-MS) to characterize their chemical profiles. In vitro assays were conducted to evaluate their anti-elastase and anti-collagenase activities, and in silico studies were performed to predict the pharmacokinetic and pharmacodynamic properties of major constituents. **Results**: GC-MS analysis showed that aldehydes, alkanes, fatty alcohols, fatty acids, and their derivatives were the predominant compound classes in both oils. Notably, oxygenated diterpenes (11.22%) were present exclusively in the MAHD oil, while triterpenoids (16.37%) were found only in the HD oil. The MAHD oil demonstrated stronger In vitro anti-elastase (IC_{50} = 42.5 μg/mL) and anti-collagenase (IC_{50} = 131.5 μg/mL) activities compared to the HD oil (IC_{50} = 66.7 and 206.8 μg/mL, respectively), reflecting an approximate 36.3% improvement in elastase inhibition and 36.4% improvement in collagenase inhibition. In silico docking indicated that the triterpenoid *β*-amyrin acetate showed the highest predicted binding affinity for porcine pancreatic elastase (PDB ID: 6QEO, ΔG = −8.1 kcal/mol) and collagenase (PDB ID: 456C, ΔG = −9.1 kcal/mol) among the major compounds analyzed. **Conclusion**: These findings demonstrate that MAHD is a greener and more efficient extraction method, yielding oil with enhanced enzyme inhibitory activity compared to conventional HD. The promising anti-elastase and anti-collagenase properties suggest that *C. olitorius* MAHD oil could serve as a potential candidate for anti-aging cosmetic formulations, following further validation.

Keywords: *Corchorus olitorius*; essential oils; microwave-assisted hydrodistillation; Gas chromatography; enzyme; cosmetic



Highlights

1. Microwave-assisted hydrodistillation (MAHD) and hydrodistillation (HD) methods pronouncedly affect the chemical composition of *C. olitorius* L. leaves essential oil.
2. The essential oils composition was identified by Gas chromatography and mass spectrometry.
3. MAHD oil possessed better quality than HD oil as it contained biologically active oxygenated diterpenes which were absent in HD oil.
4. The MAHD oil showed potential In vitro anti-elastase and anti-collagenase activities.
5. MAHD oil represents a good natural alternative to be incorporated in pharmaceutical care products and cosmetic industries.

Medical history of objective

The medicinal and culinary use of *Corchorus olitorius* is most definitively recorded in medieval Arabic and Ayurvedic texts. The 13th century physician Ibn al-Baitar, in his pharmacopeia *Al-Jami' li-Mufradat al-Adwiya wal-Aghdhiya* (compiled in 1240 C.E.), identify *C. olitorius* as both a food and medicine, prescribing it to reduce inflammation, soothe digestive ailments, and alleviate fever due to its cooling properties. Earlier, the 10th century Abbasid culinary text *Kitab al-Tabikh* by Ibn Sayyar al-Warraq documented its use in soups, suggesting widespread consumption for both nutrition and gut health in medieval Arab cuisine. In India, the 16th century's *Bhavaprakasha Nighantu* – an Ayurvedic materia medica by Bhavamisra – describes *C. olitorius* which is known as mahachanchu as a demulcent herb used in treating bile related disorders. Also, it aids in wound healing when applied topically. Across these traditions, *C. olitorius* was consistently valued for its mucilaginous texture, digestive benefits and anti-inflammatory effects. Modern research has substantially validated many of these traditional applications, revealing a broad spectrum of pharmacological effects for *C. olitorius*. These include antioxidant, anti-inflammatory properties.

Background

Essential oils produced by aromatic plants were employed for many years in food preservation and possess several pharmacological activities such as antimicrobial, analgesic, sedative, anti-inflammatory and spasmolytic activities and are largely utilized in perfume, pharmaceutical and cosmetic industries [1, 2]. They are considered as main ingredient in pharmaceutical products employed in traditional and modern medicines [3]. It is important to note that differences in climate, geographical distribution and distillation techniques severely influence the chemical composition of the essential oils leading to observed variations which in turn affect the oils biological activities [3, 4].

Hydrodistillation (HD), steam distillation and solvent extraction represent the most common methods employed in essential oil extraction [5]. Nowadays, the utilization of microwave in extraction process attracts great research interest. Microwave-assisted hydrodistillation (MAHD) represents an alternative and advanced hydrodistillation method which overcomes the drawbacks of conventional hydrodistillation [6]. MAHD has the advantages of reducing the time required for extraction and saving energy. MAHD is based on the principle of microwave energy irradiation which generates heat energy leading to accelerating the extraction process. The plant material is soaked in water and electromagnetic radiation is applied. The heat generated inside the cells leads to evaporation, expansion and increased pressure in the oil glands causing stretching out in the cell walls of the oil glands and rupturing the walls leading to increased extraction rate. Heat transfer is localised in the desired plant parts leading to oil release without excessive damage in the oil glands leading to the production of oils with high quality. However, excessive damage in the oil glands is observed in the case of hydrodistillation method [7]. In addition, it was reported that MAHD could produce a yield higher than conventional HD system by 32% [8].

Essential oils could represent an alternative, eco-friendly, safe, and affordable source for developing cosmetic formulations. Skin aging is a major global concern especially for women [9]. Serious side effects, such as irritation, hyperpigmentation and bacterial infection, arise from the employment of the available treatments like hydroxyl acids, retinoids, skin fillers, and hormone replacement therapy [10]. Given the side effects and costly anti-aging formulations, there is great demand for alternative natural sources for industrial production, such as essential oils.

Continuous waste of internal physical balance leads to the development of dermatological problems, such as skin aging [11]. The elastase enzyme is involved in the degradation of elastin, whereas the collagenase enzyme is responsible for the breakdown of collagen in the extracellular matrix, leading to skin aging [12]. Inhibition of elastase and collagenase, key enzymes involved in the degradation of skin extracellular matrix components, has been widely investigated in the development of anti-aging agents [13].

Endogenous (intrinsic aging) and exogenous factors (extrinsic aging) govern the progression of skin aging [14]. Intrinsic aging leads to a decrease in skin elasticity caused by natural long-term physical changes. Extrinsic aging is caused by toxins, chemicals, pollutants, and exaggerated exposure to UV rays from sunlight, leading to production of lipid peroxides and reactive oxygen species, causing changes in the connective tissues, disappearance of skin elasticity, and development of some skin tumors due to the increase in the levels of matrix metalloproteinases and the decrease of structural collagen and elastin [15]. Skin aging is characterized by the appearance of wrinkles, lack of elasticity, and bristling appearance [16]. It is important to note that collagen and elastin are responsible for maintaining skin cohesion and elasticity, and a decrease in their levels leads to skin aging, the appearance of wrinkles, and skin carcinogenesis [12]. Intrinsic and extrinsic aging is linked with elevated in elastase and collagenase enzyme activities [17].

The genus *Corchorus* (family Malvaceae) comprises 60 species [18]. *Corchorus olitorius* is commonly known as jute mallow or nalta jute. It is an annual herbaceous plant that grows up to 1.5 m height. The plant is cultivated in Egypt, Syria, Lebanon, Benin, Nigeria, Cameroon, Sudan, Uganda, Kenya, Zimbabwe, Brazil, India, Bangladesh, Japan, and China [19, 20]. The leaves and shoots of *C. olitorius* contain abundant proteins and minerals. The plant is employed in the form of soup in many countries because it represents a valuable source of proteins [21]. It is a familiar vegetable in Egypt. The young leaves of *C. olitorius* are a chief food employed in Egypt dating back to the time of the pharaohs [22]. The plant was traditionally used to treat aches, pains, dysentery, enteritis, fever, pectoral pains, and tumours [23]. Moreover, many reports have shown that the plant and its essential oils elicit various biological activities such as hypoglycemic, antioxidant, anti-inflammatory, analgesic, anti-tumor, anti-obesity, gastroprotective, and wound healing effects owing to the various bioactive metabolites present in the plant, such as cardiac glycosides, carotenoids, phenolics, polysaccharides, triterpenes, steroids, minerals, proteins, and vitamins [21, 24–26]. The plant was also used in the preparation of lotions, hand and facial creams, and hair tonics [21]. In addition, it was reported that the essential oil obtained from the leaves and flowers of *C. olitorius* represents a promising source of bioactive compounds that could be employed in the food and pharmaceutical industry [27]. Previous reports showed that the leaves and flowers oil exhibited antioxidant activity. In addition, it elicited antibacterial activity by showing good growth inhibition compared to the positive control. Moreover, it possessed high antimutagenic effects at 0.125, 0.0125 and 0.00125 mg/plate. Furthermore, the oil showed significant antiproliferative activity against Hela cells with IC_{50} = 4 ± 0.98 μg/mL) [25]. Reports also showed that the root essential oil exhibited antioxidant and analgesic activities [28].

To the best of our knowledge, the current work is unique in its

approach by comparing the effects of different extraction techniques (MAHD and HD) on the chemical composition of *C. olitorius* L. leaves growing in Egypt. In addition, the study investigates the anti-aging activity of both oils through the inhibition of elastase and collagenase enzymes. Also, the work provides pharmacokinetic and pharmacodynamic profiles of the major compounds present in both oils. The pharmacodynamic study was carried out to explore the mechanism and the binding modes of the tested major compounds to their receptors. Integration of both In vitro biological assays and in silico molecular docking provides a more comprehensive understanding of the bioactivity of the essential oils. This dual methodology, along with the use of novel extraction technique, provides fresh insights into the optimization of the essential oil production for medicinal use.

Methods

Plant material

The fresh leaves of *C. olitorius* growing in Egypt were collected in March 2023 from the Zoo, Giza, Egypt. The plant was kindly authenticated by Mrs. Therease Labib, the plant taxonomist at Orman botanical garden and National Gene Bank, Giza, Egypt. A voucher specimen (PHG-P-CO-338) was deposited at the botanical herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt.

Essential oils preparation for chemical analysis

Two different extraction techniques (microwave-assisted hydrodistillation and hydrodistillation) were utilized to obtain the essential oil from the plant under evaluation.

MAHD

A Smart SM-3020MS (30 L) home microwave oven was modified by making a hole at the top in the laboratory. The interior microwave cavity possessed dimensions equal to 34 × 32 × 22 cm (L × W × H). Hundred grams of the fresh leaves were introduced in 500 mL round-bottomed flask and immersed in 200 mL distilled water. The flask was connected to a Clevenger type apparatus present outside the microwave oven. The flask was subjected to heating at various powers (40 W for 15 min, 70 W for 30 min and 100 W for 30 min) [29]. The extraction was conducted using microwave mode only, with no application of grill, convection, or combination modes. The microwave-assisted extraction process is affected by the microwave power [30]. The microwave power settings of 40 W, 70 W, and 100 W were selected to balance gentle heating with efficient extraction. An initial low power setting of 40 W for 15 min was used to gradually increase temperature and avoid the degradation of thermolabile volatile compounds. This was followed by moderate (70 W) and high (100 W) power levels for 30 min each, which facilitated cell wall rupture and enhanced the release of essential oils through increased internal pressure and solvent mobility. This stepwise heating strategy aligns with previous studies: A study used the same power levels (40 W, 70 W, 100 W) to extract essential oil from *Rosmarinus officinalis*, demonstrating their effectiveness under atmospheric pressure, while another study applied the identical power sequence for the extraction of *Cestrum diurnum* essential oil using MAHD [29, 31]. These precedents support the rationale behind our approach, which aims to optimize oil yield and preserve the integrity of thermosensitive compounds. The obtained essential oils, with a yield of 0.01% (v/w), corresponding to approximately 10 mg, were stored at −4 °C for further analysis.

HD

A Clevenger apparatus was employed in the process of hydrodistillation. The extraction began by adjusting the heating mantle to rapidly raise the temperature of the mixture, bringing it to a vigorous boil (100 °C) within approximately 20 min. Once boiling started, the mantle temperature was reduced to maintain a gentle boil at around 95 °C for the remaining duration. The essential oils of the fresh leaves (100 g) were obtained after 4 h and were stored at −4 °C for analysis [32]. The yield of the essential oil was 0.01% v/w corresponding to approximately 10 mg.

Metabolic profiling of the essential oils using GC/MS

The analysis of the essential oils prepared by MAHD and HD techniques was performed using a Shimadzu GC-2010 apparatus and a quadrupole mass spectrometer Shimadzu QP-2010. Each essential oil sample was analyzed by a single injection using GC-MS. A fused bonded column (Rtx-5MS) with an internal diameter of 0.25 mm, a length of 30 m, and a film thickness of 0.25 µm was utilized. The used carrier gas was helium with a flow rate of 1.0 mL/min, the used split mode was with a split ratio = 1:15. The temperature of the injector was 250 °C, while the detector temperature was 300 °C. Initially, the column temperature was maintained isothermal for 2 min at 45 °C followed by a gradient from 45 °C to 300 °C at a rate of 5 °C/min and at the end, the temperature was maintained constant at 300 °C for 5 min. The sample (1 µL) was automatically injected using an AOC-20i auto sampler. The calculation of the peak area % was relative to the total area percent using GC solution® software ver. 2.4. GC-MS analysis was performed without the use of internal or external standards for quantification. However, an *n*-alkane series (C8–C28) was injected under the same chromatographic conditions to calculate retention indices (RIs) for each compound. The chemical components of the essential oils were identified based on the analysis of the chromatogram retention indices and mass spectral data of each peak using a computer library (NIST-11Mass Spectral Library), along with comparing these data with reported literature [33]. The relative abundances of the detected compounds were estimated based on peak area percentages, and results are therefore considered semi-quantitative.

Biological studies

The in vitro anti-elastase and anti-collagenase activities of the essential oils obtained by the two methods were assessed.

Assessment of the anti-elastase activity

The assessment of the anti-elastase activity of the essential oils was carried out as previously described by Kim et al., with minor modifications [34]. A stock solution (3.33 mg/mL) was prepared by dissolving the pancreatic porcine elastase (Sigma-Aldrich, St. Louis, MO, USA) in sterile water. The substrate *n*-succinyl-Ala–Ala–Ala–*p*-nitroanilide (≥ 98% purity, Sigma-Aldrich, St. Louis, MO, USA) was dissolved in Tris-HCL buffer (pH 8) at 1.6 mM. Incubation of the samples having concentrations ranging from 1,000 to 10 µg/mL with the prepared elastase solution in the buffer was carried out for 15 min, followed by the addition of the substrate to start the reaction. The absorbance was measured following the addition of the substrate at 381–402 nm by the mean of a microplate reader (TECAN, Inc., Durham, NC, USA). DMSO (Sigma-Aldrich, St. Louis, MO, USA) was employed as the negative control, while epigallocatechingallate (Sigma-Aldrich, St. Louis, MO, USA) was the positive control and tested at concentrations ranging from 10 to 1,000 µg/mL.

Assessment of the anti-collagenase activity

The anti-collagenase activity of the essential oils was determined as previously described by Thring et al., with minor modifications [35]. Collagenase obtained from the bacterium *Clostridium histolyticum* (ChC–EC.3.4.23.3, Sigma-Aldrich, St. Louis, MO, USA) was initially solubilized in 50 mM Tricine buffer (pH 7.5 with 10 mM $CaCl_2$ and 400 mM NaCl) to obtain 0.8 unit/mL and then added to the essential oils having concentrations ranging from 1,000 to 10 µg/mL and incubated for 15 min. The substrate *n*-[3-(2-furyl) acryloyl]-Leu–Gly–Pro–Ala (FALGPA; ≥ 98%, Sigma-Aldrich, St. Louis, MO, USA) was dissolved in Tricine buffer to a concentration of 2 mM and added to the samples. The absorbance was measured by employing a microplate reader (TECAN, Inc., Durham, NC, USA) at 335 nm. Piroxicam (≥ 98%, Sigma-Aldrich, St. Louis, MO, USA) was

employed as a positive control and tested at concentrations ranging from 10 to 1,000 µg/mL, while DMSO (Sigma-Aldrich, St. Louis, MO, USA) was used as negative control.

Statistical analysis

The assays were performed in triplicates. The values were displayed as mean ± SD. One-way ANOVA followed by Tukey's multiple comparison test was used to analyze group differences where appropriate. Differences between the IC_{50} values of the MAHD and HD oils were assessed using an unpaired Student's t-test. A *P*-value of < 0.05 was considered statistically significant. The IC_{50} was determined from the graph plots of the dose-response curve for each sample concentration by GraphPad Prism software (San Diego, CA, USA).

In silico studies

in silico pharmacokinetic and pharmacodynamic studies of the major identified compounds were carried out.

In silico pharmacokinetic study

The pharmacokinetic profile of the major identified compounds in the essential oils was determined using SWISS ADME model which is a free web tool able to predict the ADME profile (www.swissadme.ch) [36].

In silico molecular docking study

Screening of the in silico anti-elastase and anti-collagenase activities of the identified major compounds in the essential oils was carried out through the Autodock Vina platform [37]. Three independent docking runs were performed to ensure reproducibility. The crystal structures of the targets porcine pancreatic elastase (6QEO) and collagenase (456C) were obtained from the Protein Data Bank (www.rcsb.org). Notably, these enzymes have also been used in similar in silico studies with natural extracts [13]. Epigallocatechingallate (EGCG) was used as the reference drug tested against elastase target. Different studies employed EGCG as a standard and exhibited elastase inhibition with IC_{50} values equal to 93.99 and 18.2 µg/mL [12, 13]. In addition, previous reports showed that it possessed good binding affinity against the elastase enzyme (6QEO) with docking score equal to −8.80 [13]. Furthermore, piroxicam was used as the reference drug against collagenase target. Previous reports showed that it was used as standard and exhibited an IC_{50} equal to 393 µg [38]. Another study showed that the compound potently inhibited the collagenase enzyme with IC_{50} = 63 µM [39]. Validation of the docking protocol was carried out by docking the co-crystallized ligand in each receptor, then, calculation of RMSD by comparing the co-crystallized pose and the docked pose. For elastase (PDB ID: 6QEO), the grid box was centered at X = 3.377, Y = −9.618, Z = 5.656 with dimensions of 19.0389 × 21.0247 × 21.4216 A. For collagenase (PDB ID: 456C), the grid box was centered at X = −5.359, Y = 22.454, Z = 53.679 with dimensions of 33.29 × 19.40 × 22.34 A. The chemical structures of the tested compounds were drawn using ChemSketch. After drawing the structure, the ligands were exported to Open Babel to carry out energy minimization using the MMFF94 force field. The binding interactions were created through Protein-Ligand Interaction Profiler web tool (http://plip-tool.biotec.tu-dresden.de) [40]. The 3D interactions were visualized using PyMOL.

Results

Extraction time and yield

The prepared oils were yellow in color and lighter than water. No differences were observed in the yield of the prepared essential oils obtained from 100 g fresh leaves when performing the two extraction methods. The yield was the same and was equal to 0.01% v/w. The microwave-assisted hydrodistillation possessed the advantage of reducing the extraction time. The extraction process took 45 min, and the oil started to appear after 5 min from the beginning of the process. On the other hand, the HD process took 4 h, and the first droplets of the essential oil were obtained at about 30 min. Despite the widely reported efficiency of MAHD, both MAHD and conventional HD yielded the same amount of essential oil from *Corchorus olitorius* leaves. This result could be attributed to the low essential oil content of the plant material, which limits the total recoverable oil regardless of the extraction technique. Similar findings have been reported in previous studies where MAHD and HD produced comparable yields, despite significant differences in extraction time and energy input. For instance, in *Cinnamomum iners*, MAHD at 800 W for 30 min produced an identical yield (0.12%) to that obtained via 5 h of HD [41]. This finding suggests that while MAHD does not always increase oil yield, it offers clear advantages in terms of reduced extraction time, energy efficiency, and better preservation of thermolabile compounds. Thus, the use of MAHD remains beneficial, especially for applications prioritizing quality and sustainability over absolute yield.

Essential oil composition

C. olitorius has long been valued for its medicinal properties. The essential oil of the plant is traditionally extracted by hydrodistillation. However, HD is often hampered by prolonged extraction times and substantial energy demands. Microwave-assisted hydrodistillation has emerged as a promising alternative, offering potential reductions in both time and energy consumption. While studies have demonstrated that MAHD can yield essential oils with compositions comparable to those obtained via HD, its application to *C. olitorius* remains underexplored. This study aimed to fill this gap by systemically comparing the essential oil yields and compositions of *C. olitorius* leaves growing in Egypt extracted using MAHD and HD techniques, thereby providing insights into the efficacy and advantages of the MAHD method. The plant was harvested, and essential oils were extracted in March 2023.

Metabolic profiling of MAHD and HD essential oils of *C. olitorius*

A total of 43 volatile constituents belonging to different chemical classes were identified, constituting 94.73% of the essential oil prepared by MAHD (Figure 1, Figure 2), while 23 compounds were identified in the essential oil prepared by HD representing 98.82% of the oil composition (Figure 2, Figure 3). The identified compounds in both oils are listed in Table 1 and the percentage of each class present in both oils is presented in Table 2. Identification is based on comparing the mass spectral data of the compounds (MS) and their Kovats retention indices (RI) with those of NIST Mass Spectral Library, Wiley Registry of Mass Spectral Data and the reported literature.

The current study revealed that aldehydes represented 29.63 and 41.45% of the composition of the essential oils obtained by MAHD and HD respectively. Isovaleraldehyde (25.43%) represented the main aldehyde in MAHD oil, while butanal represented 40.66% of HD oil. Furthermore, alkanes represented 25.81 and 22.61% of the essential oils prepared by MAHD and HD, respectively, with 2-methyl pentane representing 14.41% of the MAHD oil, while the most predominant alkane in the HD oil was 2,2-dimethylbutane representing 11.25%. Moreover, the study showed that fatty alcohol, fatty acids and their esters represented 18.62 and 15.25% of the oil composition prepared by the two methods, respectively, with 3-hexen-1-ol representing a percentage of 6.34 and 3.26 by the two extraction methods, respectively.

It was observed that oxygenated diterpenes were present in the MAHD oil with a percentage equal to 11.22% with larixol representing 10.17%, while they were absent in the HD oil. In the same context, triterpenoids were present in HD oil having *β*-amyrin acetate with a percentage of 16.37%, while absent in MAHD oil. The presence of *β*-amyrin acetate in HD oil could be attributed to differences in the thermal profiles and extraction dynamics of the two methods. *β*-Amyrin acetate, a triterpenoid ester with a relatively high molecular weight and low volatility, is less likely to be co-distilled under rapid and intense microwave heating. In MAHD, the short exposure time and localized heating may not allow sufficient breakdown of cellular structures to release such semi-volatile compounds. In contrast, the

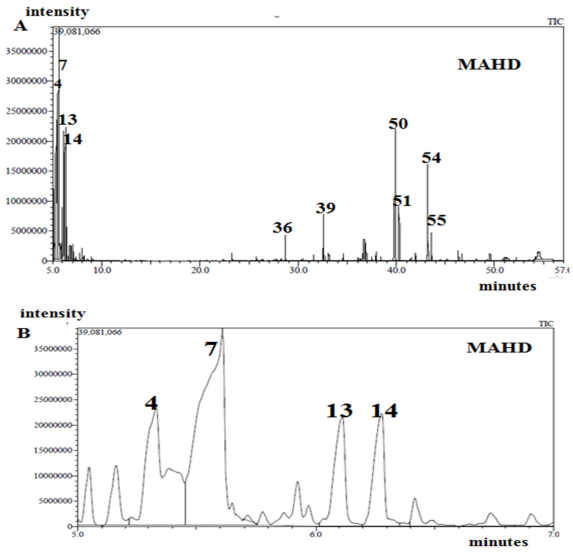


Figure 1 GC/MS analysis of essential oils from *C. olitorius* leaves extracted by MAHD technique. (A) GC/MS chromatogram of the essential oils of *C. olitorius* leaves prepared by MAHD technique. (B) Zoomed-in section of the chromatogram, highlighting the peaks eluted between 5 and 7 min. MAHD, microwave-assisted hydrodistillation; GC/MS, gas chromatography/mass spectrometry; TIC, total ion chromatogram.

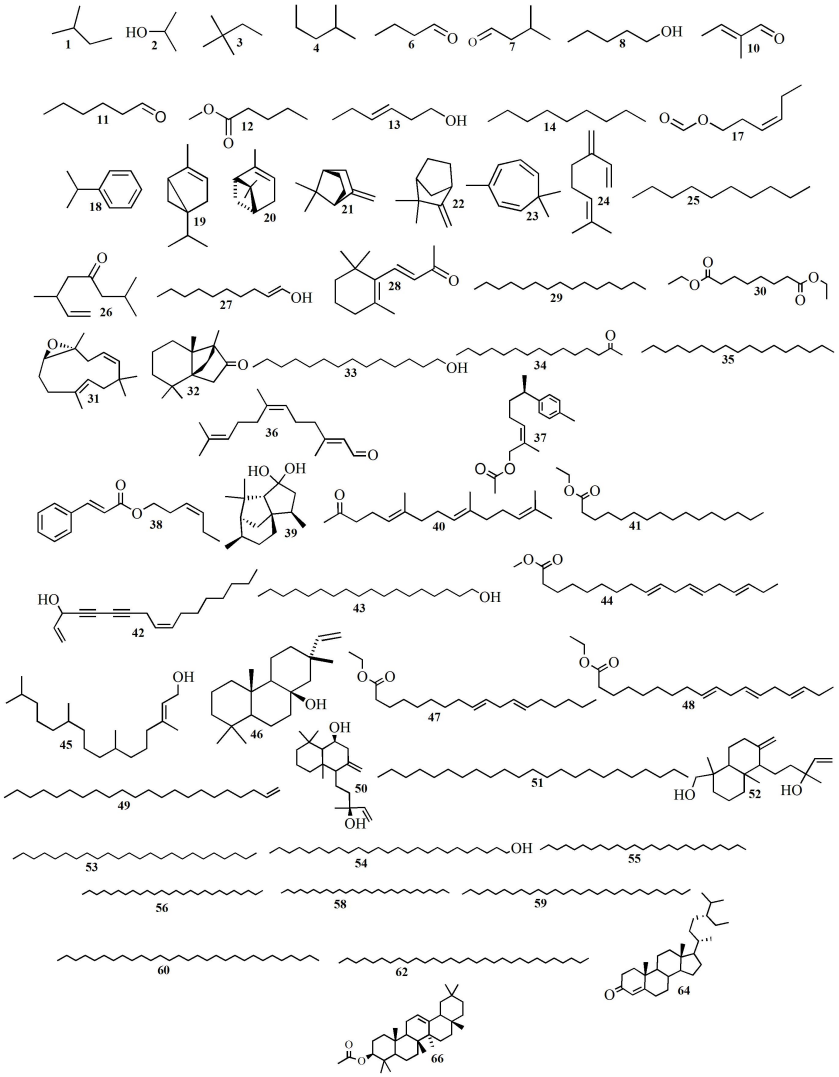


Figure 2 Chemical structures of identified compounds in MAHD and HD oils of *C. olitorius* L. MAHD, microwave-assisted hydrodistillation; HD, hydrodistillation.

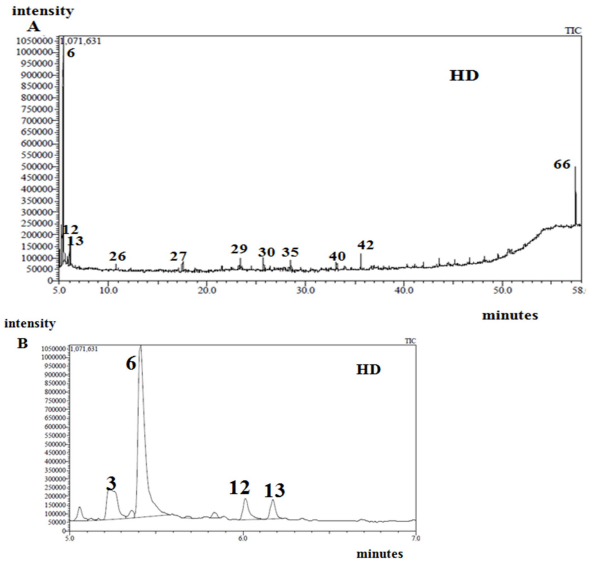


Figure 3 GC/MS chromatographic profile of *C. olitorius* leaf essential oils extracted by HD technique. (A) GC/MS chromatogram of the essential oils of *C. olitorius* leaves prepared by HD technique. (B) Zoomed-in section of the chromatogram, highlighting the peaks eluted between 5 and 7 min. HD, hydrodistillation; GC/MS, gas chromatography/mass spectrometry; TIC, total ion chromatogram.

Table 1 Chemical profile of the essential oils of *C. olitorius* leaves

Peak #	Compound	R_t	RI_{exp}	RI_{rep}	Relative %		MF	Identification
					MAHD	HD		
1	Butane, 2-methyl	5.060	468	474	/	2.23	C_5H_{12}	RI, MS
2	Isopropyl alcohol	5.169	508	510	2.77	0.46	C_3H_8O	RI, MS
3	2,2-Dimethylbutane	5.230	530	533	/	11.25	C_6H_{14}	RI, MS
4	2-Methylpentane	5.330	566	565	14.41	/	C_6H_{14}	RI, MS
5	Unidentified	5.360	577	/	/	1.20	/	/
6	Butanal	5.410	596	596	/	40.66	C_4H_8O	RI, MS
7	Isovaleraldehyde (3-methyl butanal)	5.608	669	669	25.43	/	$C_5H_{10}O$	RI, MS
8	Pentanol	5.680	694	692	/	0.30	$C_5H_{12}O$	RI, MS
9	Unidentified	5.718	709	/	0.12	/	/	/
10	Methyl-2-butenal	5.840	753	753	/	0.79	C_5H_8O	RI, MS
11	Hexanal	5.933	787	787	3.43	/	$C_6H_{10}O$	RI, MS
12	Methyl valerate	6.015	816	821	/	4.54	$C_6H_{12}O_2$	RI, MS
13	3-Hexen-1-ol	6.111	851	851	6.34	3.26	$C_6H_{12}O$	RI, MS
14	*n*-Nonane	6.275	901	900	6.27	/	C_9H_{20}	RI, MS
15	Unidentified	6.375	904	/	0.13	/	/	/
16	Unidentified	6.429	906	/	1.27	/	/	/
17	3 *Z*-Hexenyl formate	6.747	917	917	0.77	/	$C_7H_{12}O_2$	RI, MS
18	Cumene	6.916	923	930	0.53	/	C_9H_{12}	RI, MS
19	*α*-Thujene	7.035	927	927	0.62	/	$C_{10}H_{16}$	RI, MS
20	*α*-Pinene	7.155	931	932	0.33	/	$C_{10}H_{16}$	RI, MS
21	*α*-Fenchene	7.353	938	943	0.17	/	$C_{10}H_{16}$	RI, MS
22	Camphene	7.975	959	959	0.73	/	$C_{10}H_{16}$	RI, MS
23	3,7,7-Trimethyl-1,3,5-cycloheptatriene	8.175	966	970	0.28	/	$C_{10}H_{14}$	RI, MS
24	*β*-myrcene	8.914	991	991	0.21	/	$C_{10}H_{16}$	RI, MS
25	Decane	9.095	997	1,000	0.08	/	$C_{10}H_{22}$	RI, MS
26	7-Octen-4-one, 2,6-dimethyl	10.835	1,053	1,053	/	0.71	$C_{10}H_{18}O$	RI, MS
27	Decen-1-ol	17.535	1,275	1,271	/	1.41	$C_{10}H_{20}O$	RI, MS
28	*β*-ionone	23.240	1,484	1,488	0.39	0.61	$C_{13}H_{20}O$	RI, MS
29	Pentadecane	23.380	1,490	1,500	/	1.73	$C_{15}H_{32}$	RI, MS
30	Octanedioic acid, diethyl ester	25.760	1,585	1,585	/	2.46	$C_{12}H_{22}O_4$	RI, MS
31	Humulene 1, 2-epoxide	26.390	1,611	1,608	0.17	/	$C_{15}H_{24}O$	RI, MS
32	Junicedranone	27.635	1,664	1,665	0.11	/	$C_{15}H_{24}O$	RI, MS
33	*n*-Tetradecanol	27.8	1,671	1,672	0.17	/	$C_{14}H_{30}O$	RI, MS
34	*n*-Pentadecanone	28.3	1,692	1,697	0.17	/	$C_{15}H_{30}O$	RI, MS
35	Heptadecane	28.535	1,702	1,700	/	1.63	$C_{17}H_{36}$	RI, MS
36	2*E*, 6*Z* Farnesal	28.685	1,708	1,713	1.15	/	$C_{15}H_{24}O$	RI, MS

Table 1 Chemical profile of the essential oils of *C. olitorius* leaves (*continued*)

Peak #	Compound	R_t	RI_{exp}	RI_{rep}	Relative %		MF	Identification
					MAHD	HD		
37	Nuciferol acetate	31.535	1,832	1,831	0.25	/	$C_{17}H_{24}O_2$	RI, MS
38	Hexenyl cinnamate	32.48	1,882	1,881	0.59	/	$C_{15}H_{18}O_2$	RI, MS
39	Cedranediol	32.63	1,889	1,889	2.38	/	$C_{15}H_{26}O_2$	RI, MS
40	5*E*, 9*E* Farnesyl acetone	33.125	1,914	1,913	0.68	1.82	$C_{18}H_{30}O$	RI, MS
41	Ethyl hexadecanoate	34.595	1,987	1,990	0.37	/	$C_{18}H_{36}O_2$	RI, MS
42	Heptadeca-1,9-dien-4,6-diyn-3-ol	35.640	2,040	2,036	/	2.82	$C_{17}H_{24}O$	RI, MS
43	*n*-Octadecanol	36.330	2,076	2,077	0.13	/	$C_{18}H_{38}O$	RI, MS
44	Methyl linolenate	36.71	2,095	2,095	1.79	/	$C_{19}H_{32}O_2$	RI, MS
45	Phytol	36.935	2,107	2,107	0.84	/	$C_{20}H_{40}O$	RI, MS
46	Nezukol	37.515	2,139	2,133	0.21	/	$C_{20}H_{34}O$	RI, MS
47	Ethyl linoleate	37.815	2,155	2,155	0.24	/	$C_{20}H_{36}O_2$	RI, MS
48	Ethyl linolenate	37.955	2,162	2,165	0.50	/	$C_{20}H_{34}O_2$	RI, MS
49	Docosene	38.46	2,190	2,189	0.19	/	$C_{22}H_{44}$	RI, MS
50	Larixol	39.865	2,268	2,266	10.17	/	$C_{20}H_{34}O_2$	RI, MS
51	*n*-Tricosane	40.265	2,291	2,300	2.56	/	$C_{23}H_{48}$	RI, MS
52	Torulosol	41.51	2,364	2,361	0.14	/	$C_{20}H_{34}O_2$	RI, MS
53	*n*-Tetracosane	41.935	2,388	2,400	0.34	0.96	$C_{24}H_{50}$	RI, MS
54	1-Pentacosanol	43.23	2,467	2,472	6.31	/	$C_{25}H_{52}O$	RI, MS
55	*n*-Pentacosane	43.59	2,489	2,500	1.22	1.26	$C_{25}H_{52}$	RI, MS
56	Hexacosane	45.155	2,588	2,600	/	0.91	$C_{26}H_{54}$	RI, MS
57	Unidentified	46.319	2,664	/	0.43	/	/	/
58	Heptacosane	46.669	2,687	2,700	0.29	1.22	$C_{27}H_{56}$	RI, MS
59	Octacosane	48.130	2,786	2,800	/	0.72	$C_{28}H_{58}$	RI, MS
60	Nonacosane	49.547	2,886	2,886	0.32	0.70	$C_{29}H_{60}$	RI, MS
61	Unidentified	50.907	2,985	/	0.23	/	/	/
62	Triacontane	51.115	3,000	3,000	0.32	/	$C_{30}H_{62}$	RI, MS
63	Unidentified	51.285	3,013	/	0.58	/	/	/
64	Stigmast-4-en-3-one	54.235	3,234	3,237	0.59	/	$C_{29}H_{48}O$	RI, MS
65	Unidentified	54.550	3,256	/	2.27	/	/	/
66	*β*-Amyrin acetate	57.415	3,433	3,437	/	16.37	$C_{32}H_{52}O_2$	RI, MS

RI_{exp}, Kovats retention index determined experimentally on an Rtx-5 GC column relative to C8-C28 *n*-alkanes series. RI_{rep}., Kovats retention indices reported in literature and spectral databases. R_t, retention time; RI, retention index, MAHD, microwave-assisted hydrodistillation; HD, hydrodistillation; MF, molecular formula; MS, mass.

Table 2 Chemical classes of *C. olitorius* essential oils prepared by MAHD and HD

Compound class	Total peak (%) (number of compounds identified)	
	MAHD (43)	HD (23)
Monoterpenes hydrocarbons	2.34% (6)	/
Oxygenated sesquiterpenes	4.28% (5)	0.61% (1)
Oxygenated diterpenes	11.22% (3)	/
Aldehydes	29.63% (3)	41.45% (2)
Alkanes	25.81% (9)	22.61% (10)
Fatty alcohol, fatty acid and fatty acid esters	18.62% (9)	15.25% (7)
Triterpenoids	/	16.37% (1)
Steroids	0.59% (1)	/
Other classes	2.24% (7)	2.53% (2)
Total % of identified compounds	94.73% (43)	98.82% (23)

MAHD, microwave-assisted hydrodistillation; HD, hydrodistillation.

extended duration and continuous steam exposure in HD may enhance the liberation of β-amyrin acetate, potentially through thermal degradation of plant matrices or entrainment in the vapor phase, despite its limited volatility. It is important to note that β-amyrin acetate was tentatively identified based on its retention index, with comparison to NIST library entries. However, the compound was not isolated nor confirmed using ^{1}H NMR spectroscopy, and this remains a limitation of the study. Interestingly, α-amyrin acetate was previously identified in *Casia fistula* pulp oil obtained by steam distillation and represented 2.1%, further supporting the possibility of co-distillation of triterpenoids under conventional HD conditions [42]. Nevertheless, definitive confirmation of β-amyrin acetate in *C. olitorius* oil will require future work involving compound isolation and spectroscopic characterization.

Additionally, oxygenated sesquiterpenes represented 4.28% and 0.61%, respectively and monoterpenes hydrocarbons represented 2.34% in MAHD oil, while they were absent in HD oil.

Biological studies

The in vitro anti-elastase and anti-collagenase activities were investigated to explore the anti-aging potential of both oils.

Anti-elastase activity

The MAHD oil showed potential elastase inhibitory activity with IC_{50} = 42.5 ± 1.56 µg/mL, while HD oil showed moderate activity (IC_{50} = 66.7 ± 2.45 µg/mL). The standard drug epigallocatechingallate (EGCG) elicited an IC_{50} equal to 27.6 ± 1.01 µg/mL. The results are listed in Table 3 and Figure 4. The IC_{50} value of the MAHD oil against elastase was significantly lower than that of the HD oil ($P < 0.05$).

Anti-collagenase activity

The results revealed that MAHD oil showed superior inhibitory activity against collagenase enzyme than HD oil. The observed IC_{50} was equal to 131.5 ± 5.77 and 206.8 ± 9.08 µg/mL, respectively. The standard drug pyroxicam exhibited an IC_{50} equal to 63.99 ± 2.81 µg/mL (Table 3, Figure 4). The IC_{50} value of the MAHD oil against collagenase was significantly lower than that of the HD oil ($P < 0.05$).

In silico studies

Pharmacokinetic study

The results showed that butanal (6), isovaleraldehyde (7), hexanal (11), 3-hexen-1-ol (13) and larixol (50) exhibited high predicted gastrointestinal absorption owing to their reasonable solubility. Furthermore, the tested compounds showed no predicted inhibitory effect on CYP 1A2 and CYP 3A4 removing any possible interactions. The results are listed in Table 4. Moreover, the passive gastrointestinal absorption of the molecules and brain penetration were evaluated by the mean of the boiled egg (Figure 5), where the yellow area (yolk)

Table 3 In vitro anti-elastase and anti-collagenase activities of the essential oils of *C. olitorius* L. leaves

Sample	IC_{50} (µg/mL)	
	Elastase	Collagenase
MAHD	42.5 ± 1.56	131.5 ± 5.77
HD	66.7 ± 2.45	206.8 ± 9.08
EGCG (Standard)	27.6 ± 1.01	/
Piroxicam (Standard)	/	63.99 ± 2.81

The calculations were conducted in triplicate manner with expression of the results as the mean ± SD (n = 3). IC_{50} is the concentration of the tested sample at which 50% inhibition of the activity is observed. IC_{50}, half maximal inhibitory concentration; MAHD, microwave-assisted hydrodistillation; HD, hydrodistillation; EGCG, epigallocatechingallate.

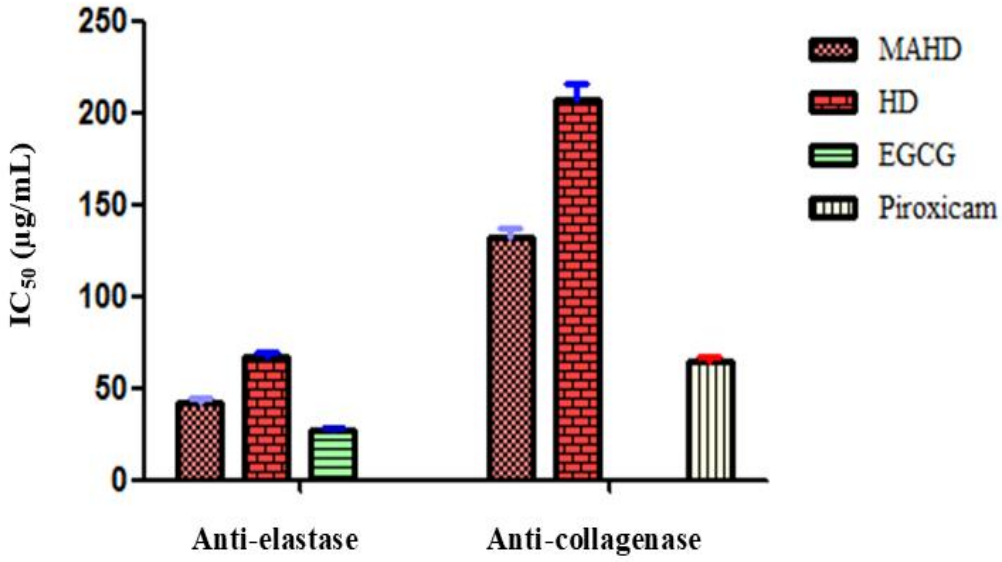


Figure 4 In vitro anti-elastase and anti-collagenase activities of the essential oils of *C. olitorius* L. leaves compared to EGCG and piroxicam. MAHD, microwave-assisted hydrodistillation; HD, hydrodistillation; EGCG, epigallocatechingallate.

Table 4 Predicted pharmacokinetic profile of the major compounds identified in the essential oils of *C. olitorius* leaves

Compound	TPSA	Log P	Solubility	GI absorption	BBB permeability	CYP 1A2 inhibition	CYP 3A4 inhibition
2,2-Dimethylbutane (3)	0.00	2.70	Soluble	Low	Yes	No	No
2-Methylpentane (4)	0.00	2.61	Soluble	Low	Yes	No	No
Butanal (6)	17.07	0.90	V. Soluble	High	Yes	No	No
Isovaleraldehyde (7)	17.07	1.14	V. Soluble	High	Yes	No	No
Hexanal (11)	17.07	1.66	V. Soluble	High	Yes	No	No
3-Hexen-1-ol (13)	20.23	1.41	V. Soluble	High	Yes	No	No
n-Nonane (14)	0.00	4.02	Soluble	Low	Yes	No	No
Larixol (50)	40.46	4.20	M. Soluble	High	Yes	No	No
1-Pentacosanol (54)	20.23	8.71	P. Soluble	Low	No	No	No
β-Amyrin acetate (66)	26.30	7.63	P. Soluble	Low	No	No	No

TPSA, topological polar surface area; Log P, logarithm of the partition coefficient; GI, gastrointestinal; BBB, blood brain barrier, CYP 1A2, cytochrome P450 1A2; CYP 3A4, cytochrome P450 3A4; V., very; M., moderately; P., poorly.

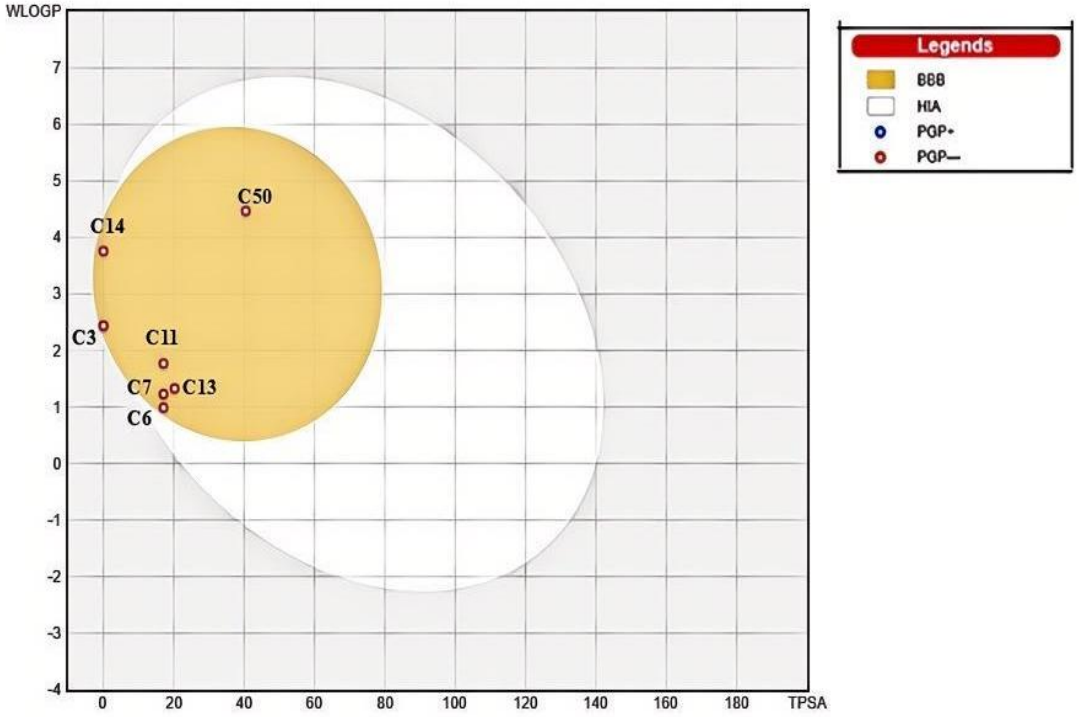


Figure 5 Boiled egg chart revealing the predicted absorption of the major compounds. BBB, blood brain barrier; HIA, human intestinal absorption; PGP, P-glycoprotein.

shows the molecules (3, 6, 7, 11, 13, 14 and 50) having high probability to penetrate the brain (BBB). Furthermore, the white region shows the molecules possessing high probability of passive human intestinal absorption. No molecules were observed in the white region. In the current study, it was observed that 7 compounds out of 10 were present in the prediction area. All the compounds are illustrated in red color showing that they are non-substrate of P-gp (PGP-).

Molecular docking study

By conducting the in vitro study of the anti-elastase and anti-collagenase activities of the essential oils of *C. olitorius* leaves prepared by MAHD and HD, it was observed that the oils elicited promising activity. The MAHD oil showed higher anti-elastase and anti-collagenase activities than the HD oil. It was deduced that the major compounds were responsible for the observed activity. Therefore, in silico study was conducted to test the effect of the major identified compounds against porcine pancreatic elastase (6QEO) and collagenase (456C). The drugs EGCG and piroxicam were used as the reference standards against the enzymes, respectively.

The results revealed that the major identified compounds prepared by MAHD and HD showed good affinities toward both enzymes with negative binding scores. β-amyrin acetate (66) showed the highest anti-elastase activity followed by larixol (50) with $\Delta G = -8.1$ and -6.3 kcal/mol, respectively, compared to standard drug EGCG having $\Delta G = -8.4$ kcal/mol. Regarding the anti-collagenase activity, it was found that β-amyrin acetate (66) exhibited the highest anti-collagenase activity among tested compounds followed by larixol (50) with binding scores equal to -9.1 and -6.9 kcal/mol, respectively, compared to the reference drug piroxicam expressing a ΔG equal to -8.9 kcal/mol. The docking results and the binding interactions of the compounds with the enzymes are listed in Table 5. The binding interactions of the standards and the compounds with the highest binding affinities to the enzymes are illustrated in Figure 6.

Table 5 Docking scores and binding interactions of the major compounds with porcine pancreatic elastase (6QEO) and collagenase (456C)

Compound name	6QEO			456C		
	Score	Hydrophobic	Hydrogen bond	Score	Hydrophobic	Hydrogen bond
2,2-Dimethylbutane (3)	−3.5	TRP 27 TRP 27 TYR 207 VAL 209	/	−4	LEU 185 VAL 219 HIS 222 TYR 244 TYR 244	/
2-Methylpentane (4)	−3.8	ILE 129 LEU 130 VAL 180 VAL 180	/	−4.3	LEU 218 VAL 219 HIS 222 LEU 239 TYR 244 TYR 244	/
Butanal (6)	−3.2	PRO 124 ARG 125 THR 128 VAL 203 ALA 208	GLN 206	−3.6	HIS 222 TYR 244	/
Isovaleraldehyde (7)	−3.7	TRP 27 TYR 207	VAL 122 VAL 209	−4.1	HIS 222 TYR 244	THR 245
Hexanal (11)	−3.5	TRP 27 TRP 27 TYR 207	VAL 122	−4.3	LEU 185 VAL 219 HIS 222	LEU 185 ALA 186
3-Hexen-1-ol (13)	−3.6	HIS 57 VAL 99 ALA 0	THR 96 THR 96	−4.1	HIS 222 TYR 244 LEU 185	/
n-Nonane (14)	−4.2	VAL 99 TRP 171 PHE 215 PHE 215	/	−4.9	LEU 185 LEU 218 HIS 222 LEU 239 TYR 244	/
Larixol (50)	−6.3	HIS 57 VAL 99 VAL 99 GLN 192 VAL 216	SER 195 SER 195	−6.9	LEU 184 LEU 185 VAL 219 HIS 222 PRO 242 TYR 244 TYR 244	/
1-Pentacosanol (54)	−5	ARG 0 VAL 99 VAL 99 TRP 171 THR 174 PHE 215 PHE 215 PHE 215	THR 96	−5.4	LEU 111 ASP 174 PHE 175 TYR 176 PHE 189 PHE 189 PRO 190 TYR 195 TYR 195 TYR 195 TYR 195	/
β-Amyrin acetate (66)	−8.1	VAL 99	THR 41 GLY 193	−9.1	MET 164 PHE 175 TYR 176 TYR 195	TYR 195
EGCG (Standard)	−8.4	VAL 99 TRP 171	ARG 0 HIS 57 THR 96 THR 96 THR 74	/	/	/
Piroxicam (Standard)	/	/	/	−8.9	HIS 222 TYR 244	HIS 222 HIS 226 PRO 242 PRO 242 ILE 243

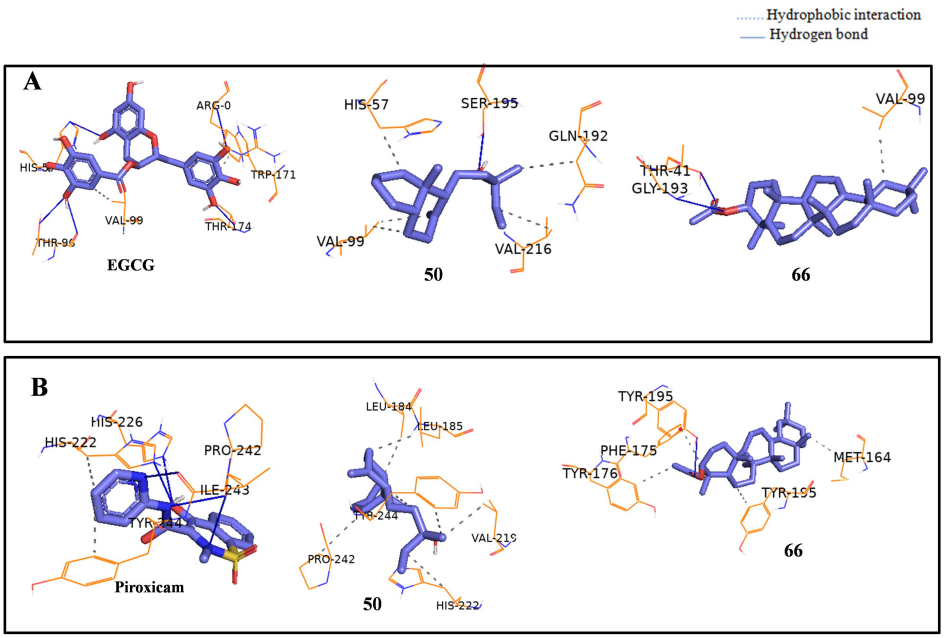


Figure 6 3D binding interactions of selected compounds with pancreatic elastase and collagenase active sites. (A) 3D binding interactions of reference drug EGCG and compounds 50 and 66 with porcine pancreatic elastase (6QEO) active site. (B) 3D binding interactions of reference drug piroxicam and compounds 50 and 66 with collagenase (456C) active site.

Discussion

Several techniques have been developed over centuries to optimize the yield, quality, and quantity of essential oils extracted from plants [4]. Hydrodistillation, steam distillation, and solvent extraction are the most common extraction of essential oil. Conventional extraction methods have some disadvantages, such as reduced extraction efficiency, decreased oil quality, loss and degradation of certain compounds, and the presence of residual toxic solvents. On the other hand, novel "green" extraction methods like supercritical fluid extraction (SFE), solvent-free microwave-assisted extraction (SFME), and ultrasound-assisted extraction (UAE), offer substantial advantages. These modern techniques are faster, more efficient, and more environmentally friendly, producing high-quality oils with minimal thermal degradation and solvent residues. Despite their benefits, some novel methods require high initial investment or specialized equipment, which can hinder their widespread adoption [43]. A previous study compared the effects of ultrasound-assisted and supercritical fluid extraction methods on the antioxidant activity of *C. olitorius* leaves growing in Iran. Caffeic acid, *p*-coumaric acid, trans-ferulic acid, quercetin, naringenin, cirsiliol, cirsilineol, quercetrin, and naringin were identified using LC-ESI-MS analysis. Moreover, the results revealed that ultrasound assisted extraction exhibited better extraction efficiency and antioxidant activity than the supercritical fluid extraction [44].

Nowadays, the use of microwave has attracted great interest in essential oil extraction, leading to an accelearation of the extraction process and energy saving [45]. While MAHD has been widely applied to extract essential oils from various botanicals, this study is the first to investigate its impact on the composition and enzyme inhibitory activities of *C. olitorius* essential oil [29, 31]. In this study, it was deduced that MAHD oil possesses higher quality than HD oil owing to the presence of higher-level oxygenated compounds [46]. In the same context, triterpenoids were present in HD oil with high percentage of *β*-amyrin acetate, whereas they were absent in MAHD oil. Previous reports revealed that α-amyrin acetate was identified in *Casia fistula* pulp oil using GC/MS analysis [42].

The observed variation in the chemical composition of both oils is attributed to the different extraction techniques, which affect the quality and composition of the oils [47]. Moreover, differences were observed between the essential oil composition of *C. olitorius* L. leaves growing in Egypt and previous reports owing to different geographic regions, soil composition, and climate [48]. Previous studies have shown that hexadecanoic acid is the major compound in the dry oil obtained from the leaves of *C. olitorius* growing in Riyadh, while ethyl palmitate is the most predominant in the stems [21]. In another study, nonadecane and heneicosane were found to be the major compounds present in the essential oil prepared by hydrodistillation of the leaves and flowers of the plant growing in Tunisia [25]. In addition, it was previously reported that 2-hexanone, 6-(acetyloxy)-(29.25%), 2 pentanone, 4-hydroxy-4-methyl-(20.33%), propane-1,1dioldiacetate (14.24%), and acetohydroxamic acid (12.27%) were the major compounds identified in the essential oil obtained by hydrodistillation of *C. olitorius* fruits growing in India [27]. In addition, previous reports showed that the leaves essential oils possessed analgesic activity owing to the presence of alcoholic, terpenic, phenolic or ketonic compounds and the possibility of a synergistic activity between the active constituents [28].

By reviewing the literature, it was observed that *C. olitorius* is a dietary source rich in phenolic compounds that may exert anti-aging effects [49]. It was reported that the aqueous fraction of the plant at a concentration of 20 μg/mL re-established the production of collagen, which decreased after exposure to UV irradiation [50]. Moreover, reports have shown that the major identified compounds in the current study possessed promising antioxidant and anti-inflammatory activities. Therefore, it was reasonable to assess the anti-aging activity of both oils through integrated In vitro and in silico approaches.

Previous studies have shown that isovaleraldehyde is an aldehyde volatile compound present in coffee that participate in the antioxidant activity of coffee [51]. The results of this study revealed that oxygenated diterpenes represented (11.22%) of the MAHD oil composition and were absent in HD oil. Therefore, the level of oxygenated compounds in MAHD oil was higher than that in HD oil, explaining its better quality and therapeutic activity [46]. Previous reports have shown that diterpenes possess promising antioxidant, radical-scavenging, anti-inflammatory, and anti-elastase activities [52, 53]. A European patent (EP2853254A1) proposed the use of larixol in cosmetic preparation used for skin lightening as its main ingredient. Larixol exerts strong anti-inflammatory activity by inhibiting

fMLP-induced superoxide anion production and neutrophil chemotaxis [54]. This anti-inflammatory activity is mechanistically relevant for skin health, as inflammation and oxidative stress are contributors to skin aging. Furthermore, 3-hexenol with a percentage of 12.95% was identified in the essential oil prepared from the leaves of *Forsythia koreana* and contributed to its promising antioxidant activity [55]. In addition, it was previously reported that *β*-amyrin and *α*-amyrin acetate possessed potent anti-inflammatory activities [56].

The current study showed that MAHD oil exhibited higher anti-elastase and anti-collagenase activities compared to the HD oil. The findings showed that isovaleraldehyde (25.43%), 2-methyl pentane (14.41%), larixol (10.17%), 1-pentacosanol (6.31%) and *n*-nonane (6.27%) were present in MAHD oil and absent in HD oil. HD oil showed the presence of *β*-amyrin acetate (16.37%). Moreover, it was observed that 3-hexen-1-ol was present with both extraction methods but with higher percentage in MAHD oil. The higher activity of the MAHD oil could be attributed to the presence of the previously mentioned compounds in MAHD oil comprising the oxygenated diterpenes and to the synergistic activity between the oil components [57].

Although the GC-MS analysis provided detailed information on the chemical composition of *C. olitorius* essential oil, it should be noted that the major compounds were not confirmed using authentic standards. The identifications are therefore tentative, based on high spectral match factors (≥ 90%) with the NIST library and with previously reported profiles. Future research should include the use of authentic standards to verify compound identity and purity, which would strengthen the reliability of these findings.

The observed in silico activity of the docked compounds is related mainly to the hydrophobic interactions with the amino acid residues present in the enzymes due to the hydrophobic nature of the tested compounds. The hydrophobic interactions play a pivotal role in establishing the binding affinity between the compound and the receptor and determine the selectivity of the drugs toward the target [58].

The observed in vitro anti-elastase and anti-collagenase activities of *C. olitorius* essential oils are in line with previous reports demonstrating similar enzyme inhibitory effects from plant-derived extracts. For instance, an In vitro study investigated the *n*-hexane soluble fraction of the methanol leaf extract of *Stenocarpus sinuatus* and reported significant inhibitory activities against elastase and collagenase supporting its potential as an anti-aging agent [13]. Such findings highlight the relevance of targeting these enzymes as a valid approach for assessing the anti-aging potential of plant extracts. The comparable enzyme inhibition profiles in our study suggest that *C. olitorius* essential oils, particularly the MAHD oil, may likewise hold promise for further development of anti-aging cosmetic formulations.

The molecular docking results obtained in this study were in agreement with the In vitro enzyme inhibition data, suggesting that key constituents of the essential oil may contribute to the observed bioactivity through direct interactions with the target proteins. However, as emphasized in previous reports, in silico models such as molecular docking are predictive tools that provide insight into potential binding modes and affinities, but they possess important limitations. Docking depends on static three-dimensional structures of proteins and ligands, assumes rigid binding sites, and often uses simplified scoring functions that may not accurately reflect the complex physicochemical interactions occurring inside the biological systems. Additionally, such models do not account for crucial pharmacokinetic and pharmacodynamic factors, such as absorption, metabolism and cellular uptake [59]. Therefore, while the docking data supports the plausibility of interaction at the molecular level, it should be interpreted with caution and considered hypothesis-generating rather than confirmatory. Further experimental validation is necessary to fully elucidate the bioactivity and mechanism of action of the identified compounds.

Although the current study suggests a correlation between the presence of specific compounds in the tested essential oils and the observed anti-elastase and anti-collagenase activities, it is important to approach the findings with caution when interpreting them as definitive causal relationships. The observed biological activity may be influenced by a combination of factors, including the interactions between different compounds present in the oil, rather than being solely attributable to a single specific compound [57]. Furthermore, although correlations provide useful insights into potential mechanisms of action, they do not establish a direct cause-and-effect relationship. To validate these findings and establish causality, future studies should focus on isolating and testing individual compounds in the oil. By doing so, we could determine whether a particular compound or a combination of compounds is primarily responsible for the observed biological activity. In addition, employing more detailed mechanistic studies could provide deeper insights into the specific interactions responsible for the observed effects.

This study focused on the MAHD and HD oils obtained from the leaves of *C. olitorius* growing in Egypt. The findings revealed the promising enzyme inhibitory activity of MAHD oil from this specific source, generalizing these results to the plant growing in other regions may require further investigation. Environmental factors, such as soil composition, climate, and cultivation practices, can affect the chemical composition and biological activity of the plant [48]. Therefore, the observed effects may be unique to the Egyptian-grown plant. To assess the global applicability of these findings, future studies should explore the In vitro enzyme inhibitory activity of the plant grown in different geographical locations. This will help determine whether biological processes are consistent across regions or are influenced by local environmental factors.

Despite the encouraging results, this study possesses certain limitations that should be acknowledged. Only two extraction methods, MAHD and HD were employed, which may not fully show the complete range of bioactive constituents present in *C. olitorius* leaves. Moreover, the enzyme inhibitory activities were assessed only through In vitro assays, which do not fully replicate the complexity of biological systems in vivo. The in silico molecular docking provided insights into the possible binding interactions; however, such computational predictions require further experimental validation under physiological conditions. In addition, the study did not investigate the cytotoxic activity of the oils, skin permeability, or stability, which are critical for their practical application in anti-aging or cosmeceutical formulations. Therefore, future work should focus on optimizing extraction techniques, confirming bioactivities through cell-based and in vivo models, and evaluating the safety profile of *C. olitorius* essential oils.

Conclusions

The different extraction processes are considered among the factors which influence the chemical composition of the essential oils leading to variations in the biological activities. Currently, the use of microwave in extraction process is very beneficial as it represents an eco-friendly technique. Additionally, it saves time and energy. In the present study, MAHD and HD techniques were employed to extract the essential oils from *C. olitorius* L. leaves growing in Egypt. Based on the study results, it was deduced that MAHD oil possessed better quality than HD oil as it contained high level of biologically active oxygenated diterpenes which were absent in HD oil. Moreover, MAHD oil exhibited potential elastase and collagenase inhibitory activities owing to the major identified compounds and to the synergistic activity between the oil components. Furthermore, the results of the in silico molecular docking showed good binding affinities of the major identified compounds with elastase and collagenase enzymes which support the assumption that the major compounds are responsible for the observed activities. While, the results suggest that the combined action of these compounds may contribute to the observed biological activity, the specific nature of these synergistic interactions remains to be fully elucidated. Further research is needed to identify how the individual components interact with each other at the molecular level and how these interactions enhance or modify the overall effect of the

oil. Understanding these interactions will be crucial in determining whether the oil's therapeutic potential arises from the combined action of multiple compounds or from a few key bioactive substances. The potential application of MAHD oil in cosmetic formulations remains a hypothesis at this stage. The absence of toxicity assessments, in vivo studies, and mechanistic investigations limits the ability to make definitive claims. Thus, while the study offers promising evidence of the potential biological activity of MAHD oil, as it represents a good natural alternative anti-aging candidate that could be employed in developing pharmaceutical skin care products and cosmetics, further research is essential to confirm the safety and the causal role of the individual components and to refine our understanding of how the oil exert its effects.

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