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Research article

Phytochemical characterization, antimicrobial properties and *in silico* modeling perspectives of *Anacyclus pyrethrum* essential oil

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ABSTRACT

Medicinal plants are used widely in the treatment of various infectious diseases. One of these medical plants is Moroccan plants such as *Anacyclus pyrethrum*. In this study, the essential oil isolated from the leaves of *Anacyclus pyrethrum* (APEO) by the hydrodistillation method was analyzed using (GC/MS) analysis. A total of forty-four compounds were identified form the oil and the oxygenated monoterpenes were the most abundant class of compounds. The major identified compound is santolina alcohol (40.7 %), followed by germacrene-D (8.9 %). The *in-vitro* assessment of the antimicrobial efficacy of APEO encompassed an investigation involving six microbial strains, including two Gram-positive bacteria, four Gram-negative bacteria, and three fungal strains. The findings revealed noteworthy antibacterial and antifungal properties against

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all examined microorganisms, with inhibitory zone diameters ranging from 25.67 ± 0.06 mm to 25.19 ± 0.03 mm for Gram-positive bacteria and from 22.34 ± 0.01 mm to 14.43 ± 0.02 mm for Gram-negative bacteria, as determined through the disc-diffusion assay. In the case of antifungal activity, inhibitory zones ranged from 24.57 ± 0.04 mm to 18.37 ± 0.06 mm. Further evaluation revealed that the MIC values of Gram-positive bacteria were at the concentration 0.25 % v/v, while MBC values ranged from 0.25 % to 1.0 % v/v. The Gram-negative bacteria exhibited MIC values spanning from 0.5 % to 2.0 % v/v, with MBC values in the range of 0.5 %–2.0 % v/v. For the fungal strains, MIC values ranged from 0.5 % to 1.0 % v/v, while the MFC consistently remained at 1.0 % for all tested fungal strains. The assessment of the MBC/MIC and MFC/MIC ratios collectively indicates that *A. pyrethrum* EO possesses bactericidal and fungicidal attributes. The *in silico* study of bioavailability predictions for compounds in APEO based on six physicochemical properties show optimal physiochemical properties including size, lipophilicity, solubility, flexibility, and saturation. α-Pinene, limonene, germacrene D, and (E)-β-farnesene are nonpolar due to their lack of polar groups, and the ADME profile indicates desirable properties for considering these compounds in drug development. Molecular docking investigation indicates that all the compounds of APEO reside well into the binding site of the DNA gyrase B enzyme of *Staphylococcus aureus* by mediating a number of significant interactions with the binding site residues. The ADME analysis suggested that the major compounds APEO possess desirable properties for further consideration in drug development. In light of these findings, APEO could serve as a natural source for the elaboration of new and active antimicrobial drugs.

1. Introduction

Plants have been employed for the management of various ailments since ancient times and continue to serve as a significant source of pharmaceutical compounds in the modern era [1–3]. Medicinal plants include a wide range of bioactive phytochemicals or bionutrients. Recent research highlighted many classes of these bioactive substances, including phenolics, alkaloids, terpenoids, phytosterols, sulfides, and thiols, for their potential in treating chronic ailments such as cancer, diabetes, coronary heart disease, and infections. Each class of these effective agents includes a wide range of different compounds, many of which demonstrate several physiological activities [4–8]. The World Health Organization (WHO) has identified a global priority pathogen list that includes multidrug-resistant bacteria such as *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, particularly methicillin-resistant *Staphylococcus aureus* (MRSA), certain Enterobacteriaceae, *Serratia marcescens*, *Enterococci* spp., and *Stenotrophomonas maltophilia* and encouraged the scientific community is to intensify efforts to control these pathogens [1]. Also, fungal infections have recently become a widespread health issue that affects people with weakened immune systems as well as those who are generally healthy [9]. Unfortunately, the growing prevalence of antibiotic-resistant bacteria globally threatens antibiotic efficacy, worsened by insufficient efforts from pharmaceutical companies in developing new antibiotics [10]. Hence, Medicinal plants, which contain a wide variety of renewable bioactive molecules, show potential as a promising source for new antimicrobial treatments [11]. However, most global pharmacopeias discourage the utilization of plant-derived medications lacking established medicinal efficacy. Certain developed nations, such as the United Kingdom, and Germany, maintain distinct herbal pharmacopeias. However, in practical terms, a considerable quantity of unofficial medicinal compounds is consistently employed [12]. Therefore, the scientific exploration of medicinal plants holds considerable significance in the isolation of bioactive compounds [13], which can potentially be formulated into clinical agents. These agents may exist in their natural form or may undergo synthetic modifications, resulting in synthesized analogs that exhibit enhanced clinical efficacy or reduced adverse side effects [14].

Essential oils (EOs) play a crucial role in drug discovery due to their rich chemical composition, which often includes bioactive compounds with therapeutic potential [15]. Numerous studies highlight the significance of essential oils from various plant sources in providing a valuable reservoir for identifying and developing novel pharmaceutical agents [16]. The discovery of antimicrobial agents from natural sources, particularly essential oils, is of great importance, especially in the era of antibiotic resistance [17]. Essential oils have demonstrated potent antimicrobial activity, and their exploration has emerged as a promising avenue for combating infectious diseases [18].

Anacyclus pyrethrum (L.) belongs to the Asteraceae family and is widely spread in Morocco and Algeria. This plant species is characterized by the presence of two varieties, namely *Anacyclus pyrethrum* var. *Pyrethrum* (L) and *Anacyclus pyrethrum* var. *depressus* (Ball) Maire [19]. *Anacyclus pyrethrum* is widely used in traditional medicine for addressing various conditions, such as antibacterial, antiviral, anti-catarrh, anti-rheumatic, analgesic, carminative, digestive improvement, emmenagogue, febrifuge and vermifuge [20]. Traditional medicine predominantly employs roots, although other plant parts are also utilized [20,21]. In the scientific literature, diverse biological activities have been documented, encompassing Anti-inflammatory, and Antioxidant [22], antimicrobial [23], insecticidal [24], antidepressant [25], antidiabetic [26], anesthetic [27], anticancer [28], immunomodulatory [29], and help stimulate saliva production [30]. Therefore, the scientific verification of this plant holds significant scientific value.

In the current work, we aimed to report the volatile content of *A. pyrethrum* essential oil (APEO) and evaluate its antibacterial and antifungal effects. Parallel to this *in vitro* research, computational exploration was conducted, incorporating molecular docking, a method in drug discovery that predicts and analyzes interactions between molecules (ligands) and target biomolecules (receptors or enzymes). Additionally, analysis of the absorption, distribution, metabolism, excretion, and toxicity (ADME) was also employed in the

computational investigation.

2. Materials and methods

2.1. Plant material

Anacyclus pyrethrum leaves were collected in August 2023 from Oukaïmeden, High Atlas of Morocco (31°01′ N 07°51′ W). The plant was identified taxonomically by a botanist from the Unit of Botany at the University of Sidi Mohamed Ben Abdellah, Morocco. A voucher specimen was then given (BLUMP 507). Plant leaves were air-dried at adequate conditions.

2.2. Essential oil isolation

The dried leaves (150 g) were subjected to hydrodistillation using a Clevenger-type glass device for 4 h. The oils were collected in glass vials, weighed, and preserved at 4 °C for upcoming analysis. This experiment was conducted three times.

2.3. Chromatographic analysis of the essential oil

The obtained Essential oil samples were examined with Gas chromatography coupled with mass spectrometry (GC/MS) using a Shimadzu GC/MS-QP 2010 (Koyoto, Japan) united to a mass spectrometer (SSQ 7000 quadrupole: Thermo-Finnigan, Bremen, Germany). The used capillary column was Rtx-5MS (30 m length, 0.25 mm internal diameter and 0.25 μm film thickness, USA). The run started with a temperature of 45 °C for 2 min followed by gradient increment to 300 °C. The injector's temperature was held at 250 °C while the detector's temperature was kept at 280 °C. Automatic sample injection was conducted (1 μL, split ratio of 1:15) and Helium gas was the carrier gas used with a flow rate of 1.41 mL/min. The mass spectrometry was performed by utilizing the following conditions: ion source temperature was set at 200 °C, ionization voltage of 70 eV was applied, and the scan range was performed from 35 to 500 amu.

2.4. Identification of oil components

GC peaks mass spectra were identified by searching the commercially available libraries (WILEY, NIST), the compounds identification was further confirmed by calculating the retention indices (RI) of the peaks compared to (C6 –C22) *n*-alkanes, and then matching with the published data. Quantification was performed by peak area percentage and reported as an average of three measurements [31].

2.5. Microorganisms

In this investigation, a total of six bacterial strains were employed. These strains comprised four Gram-negative bacterial species; *Escherichia coli* ATCC 25922, *Proteus mirabilis* ATCC 25933, *Salmonella Typhimurium* ATCC 700408, and *Pseudomonas aeruginosa* 27853. Additionally, two Gram-positive bacterial species, namely *Staphylococcus aureus* ATCC 29213 and *Listeria monocytogenes* ATCC 13932, were included in the study. Furthermore, the study encompassed three fungal strains, including a yeast species *Candida albicans* and dermatophyte fungus *Trichophyton rubrum*, both of which were sourced from clinical isolates. Additionally, a strain of *Aspergillus niger*, isolated from food spoilage, was incorporated. All the microbial strains utilized in this investigation were procured from the Laboratory of Microbial Biotechnology and Bioactive Molecules, situated at the Faculty of Sciences, Fez, Morocco.

2.6. Disc-diffusion test

The antimicrobial efficacy of *A. pyrethrum* essential oil was assessed utilizing the agar disc-diffusion method, with slight adjustments as outlined in Ref. [32]. In a concise summary, the microbial culture suspensions were inoculated onto Yeast Extract-Peptone-Dextrose Agar (YPD agar) for fungi and Luria–Bertani (LB agar) medium for bacteria. Preceding their placement onto agar plates, sterile paper discs with a diameter of 6 mm were impregnated with 10 μL of pure essential oil of *A. pyrethrum*. For bacterial assessments, positive controls consisted of chloramphenicol (10 μg/disc), while Nystatin (10 μg/disc) served as the positive control for fungal evaluations. Incubation periods for bacteria were set at 24 h at a temperature range of 35 °C, whereas fungi were incubated on plates for 48–72 h at 25 °C. Following incubation, the diameters of the inhibitory zones were measured in millimeters, and the results were presented as the mean ± standard error of means based on three independent trials.

2.7. MIC test

The minimum inhibitory concentration (MIC) of APEO was determined using a previously described method with slight changes [1]. In brief, EOs were serially diluted in sterile 96-well plates containing Mueller–Hinton broth with 2 % dimethyl sulfoxide (DMSO), ranging from 8.0 % to 0.007 % (v/v) using a two-fold dilution scheme. It is important to note that prior research has confirmed that concentrations of DMSO up to 7.8 % have no significant impact on viable microbial cell counts [33]. Subsequently, 10 μL of the pre-prepared microbial culture was inoculated into each well. The 96-well plates were then incubated overnight at temperatures

between 30 and 35 °C for bacteria or at 20–25 °C for fungi. The bacterial medium was replaced with peptone yeast extract broth for fungal strains, and incubation was carried out for 48 h at 25 °C. As a control for standard growth, Mueller–Hinton broth or YPD broth containing 5 % DMSO without any microbial suspension was used. Following incubation, 95 % *p*-iodonitrotetrazolium chloride (TTC) was added to all microtubes to assess microbial growth through the detection of a color shift from yellow to pink. The change in color is typically observed after a period of 30 min of incubation. This provides sufficient time for the viable bacteria to convert TTC into the formazan product, leading to a noticeable shift in color that can be used to estimate the minimum inhibitory concentration (MIC). The MIC was defined as the highest dilution of the sample at which this color shift was no longer observable [1].

2.8. MBC and MFC tests

The study also involved an assessment of the Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) [3]. To summarize the methodology, 50 μL of liquid samples derived from the 96-wells (of MIC test) that displayed no observable microbial growth in the Minimum Inhibitory Concentration (MIC) assay were aseptically transferred onto the surface of

Table 1
Volatile compounds identified in APEO.

No.	*KI	Compounds	Relative Abundance %
1.	939	α-pinene	5.7
2.	954	Camphene	0.2
3.	971	Sabinene	0.1
4.	979	β-pinene	0.3
5.	982	Myrecene	0.5
6.	986	Yomogi alcohol	3.0
7.	1025	*p*-Cymene	0.1
8.	1028	ᴅ-Limonene	3.1
9.	1030	1,8-cineole	0.3
10.	1037	**Santolina alcohol**	**40.7**
11.	1080	Artemesia alcohol	4.3
12.	1121	Campholenal	0.1
13.	1139	(E)- Pinocarveol	1.3
14.	1146	Camphor	2.5
15.	1155	Pinocarvone	1.9
16.	1166	Borneol	0.3
17.	–	Santolina acetate	2.2
18.	1181	Terpenen4-ol	0.3
19.	1199	α-Terpineol	0.2
20.	–	Hexyl isovalerate	0.1
21.	1289	Bornyl acetate	1.0
22.	1340	δ -Elemene	Tr
23.	–	Silpheporfolene	Tr
24.	–	(Z)-Jasmone	Tr
25.		Phenethyl acetate	0.1
26.	1377	α-Copaene	0.1
27.	–	β -Maaliene	0.4
28.	1392	β -Elemene	0.6
29.	1419	β-Caryophyllene	1.7
30.	1456	(E) - β -Farnesene	4.0
31.	1480	α-Humulene	0.4
32.	1485	**Germacrene-D**	**8.9**
33.	1502	Bicyclogermacrene	0.2
34.	1503	α-Muurolene	0.8
35.	1520	δ-Cadinene	1.1
36.	1536	(Z)-Nerolidol	0.3
37.	1570	Germacrene-D-4-ol	1.6
38.	1579	Caryophyllene oxide	0.9
39.	–	Caryophylladienol	2.8
40.	–	epi-α-Cadinol	1.2
41.	–	epi-α -Muurolol	1.6
42.	1639	α -Muurolol	0.6
43.	1650	α-Cadinol	0.4
44.	1670	β -Bisabolol	Tr
Monoterpene hydrocarbons		10 %	
Oxygenated Monoterpenes		58.3 %	
Sesquiterpene hydrocarbons		18.2 %	
Oxygenated Sesquiterpenes		9.4 %	
Total identified		95.9 %	

Constituents were determined based on their mass spectra and kovats indices (KI). The presented data are the average of three replicas. KI: Kovats index; (Tr): Traces.

agar plates containing Luria-Bertani (LB) agar for bacterial strains or Yeast Peptone Dextrose (YPD) agar for fungal strains. Subsequently, these agar plates were incubated under conditions specific to each microorganism, including the appropriate temperature and incubation duration. Following the designated incubation period, the extent of microbial growth on the agar plates was assessed. The MBC or MFC was determined as the concentration level at which no discernible bacterial or yeast colonies were observed on the agar medium plate. Moreover, we computed the ratios of MBC to MIC and MFC to MIC to gain insights into the potential mechanism of action of the substance being investigated.

2.9. In silico ADME analysis

The analysis of physiochemical properties and pharmacokinetics of compounds present in APEO were assessed by SwissADME, an online web server designed for the comprehensive analysis of ADME (Absorption, Distribution, Metabolism, and Excretion) profile of the chemical compounds [34]. For this analysis, the seven compounds with the highest peak areas were specifically chosen. Utilizing the Simplified Molecular Input Line Entry System (SMILES) notation, the selected compounds were represented and used as inputs for the SwissADME analysis.

2.10. Molecular docking

In order to rationalize the plausible mechanism of the observed antibacterial activity of A. pyrethrum essential oil, molecular docking simulation was employed. The compounds namely, santolina alcohol, α-piene, yomogi alcohol, limonene, E-β-farnesene, germacrene and artemisinic alcohol were selected for the docking studies. The compounds were sketched in MOE v.2019.01 software and subjected to preparation [35]. The preparation involves the correction of formal charges and the addition of hydrogens. The prepared compounds were energy-minimized using the MMF94x force field. *Staphylococcus aureus* DNA Gyrase B was selected as a protein target. The crystal structure of the target protein was obtained from Protein DataBank with the PDB ID 3G7B [36]. The structure was subjected to structure correction and protonation using the Structure Preparation wizard of MOE. Subsequently, the structure was subjected to energy minimization using the Amber10: EHT force field. The coordinates of the cognate ligands were defined as a docking grid and all the compounds were docked to the defined grid. The Triangular Matcher was kept as a placement method with London dG and WSA/GBVI dG as scoring and rescoring functions. For each compound fifteen poses were generated and five were retained for analysis. The interactions were visualized by Chimera software.

2.11. Statistical analysis

All assays were carried out in three separate experiments, and the resulting data are established as mean ± SD. Statistical analysis was performed using GraphPad 9, and mean comparisons were accomplished with one-way analysis of variance (ANOVA) followed by the Tukey test.

3. Results and discussion

3.1. GC-MS analysis of the essential oil

The essential oil of *A. pyrethrum* was isolated from the fresh leaves by hydro-distillation method using a Clevenger-type apparatus. The chemical composition of the isolated essential oil was determined by GC/MS analysis. Forty-four components representing 95.9 % of the leaves' essential oil were identified (as shown in Table 1 and Fig. 1).

Oxygenated monoterpenes represent the main class of compounds in APEO accounting for (58.3 %). Santolina alcohol (40.7 %), germacrene-D (8.9 %), and α-pinene (5.7 %) were the main identified components. This present result is different from that was reported previously in the literature. Oxygenated sesquiterpenes were reported as the most abundant class of essential oils collected from

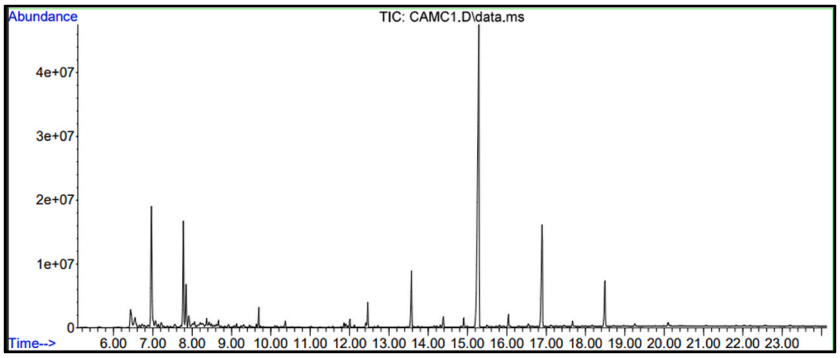

Fig. 1. Chromatogram of GC-MS analysis of APEO.

the aerial parts at different stages of the vegetative cycle of Algerian *A. pyrethrum* (Selles et al., 2013). Moreover, Elazzouzi et al. studied the essential oil in the leaves of the Moroccan *A. pyrethrum* and reported that oxygenated sesquiterpenes are the major class of compounds in leaves' essential oil. Additionally, they reported that the major components in the leaf oil were spathulenol (16.9 %) and caryophyllene oxide (7.11 %) [19]. On the other hand, spathulenol was not detected among the components of the isolated essential oil in our current study, however, caryophyllene oxide was identified but with a minor abundance (0.9 %).

Germacrene-D was reported as the major component of the essential oil isolated from *A. pyrethrum* cultivated in Algeria [23]. Germacrene-D is detected in our results as a major component of the oil which is in line with the results reported from EO isolated from *Anacyclus monanthos* [37].

Trans-chrysanthenyl acetate was also reported from Tunisian *A. clavatus* species as the major constituent with a percent composition of (12.3 %) [38]. While, the main compound identified in the essential oil of *Anacyclus cyrtolepidioïdes* species collected from Tunisia was α-pinene [39] which is also identified in our study from APEO with a percent composition of 5.4 %.

These differences in the composition of the essential oil may be attributed to some factors like the regional climatic variation, period of harvesting as well as environmental conditions and the method of oil extraction which leads to significant effects on the volatile oil profiles [40].

In our study, the unique climatic conditions of Oukaïmeden in the High Atlas of Morocco, along with the harvesting period in August, likely contributed to the observed differences in the essential oil composition compared to the same plant collected in Algeria [41]. These factors highlight the natural variability in essential oil profiles, offering valuable insights into how environmental conditions can influence chemical composition. However, they also pose challenges for reproducibility and direct comparison with other studies. To address this, future research could benefit from more standardized protocols to minimize these variations and enhance comparability.

3.2. Antimicrobial effect of the essential oil

The antimicrobial efficacy of APEO is demonstrated in Fig. 2, with the assessment primarily reliant on the measurement of the inhibitory zone diameter. Among the various microbial strains tested, the bacterium that demonstrated the highest susceptibility to APEO was *S. aureus* (25.67 ± 0.06 mm), followed by *L. monocytogenes* (25.19 ± 0.03 mm), *P. mirabilis* (22.34 ± 0.01 mm), S. Typhimurium (19.29 ± 0.03 mm), and *E. coli* (19.22 ± 0.06 mm), whereas *P. aeruginosa* exhibits the lowest susceptibility with a diameter of 14.43 ± 0.02 mm. The Gram-positive bacteria were more susceptible than the Gram-negative. The Gram-positive bacteria have a thick peptidoglycan layer, rendering them more susceptible to a wide range of antibacterial agents. Gram-negative bacteria, on the other hand, possess a thinner peptidoglycan layer but have an outer membrane containing lipopolysaccharides. The presence of this outer layer acts as an additional barrier, making it more challenging for treatments to penetrate [42,43]. On the other side, with respect to fungal strains, *Candida albicans* is notably the most susceptible, displaying an inhibitory zone diameter of 24.57 ± 0.04 mm, followed by *Aspergillus niger* (21.53 ± 0.08 mm). Whereas, *Trichophyton rubrum* manifests the least susceptibility, as indicated by an inhibitory zone diameter of 18.37 ± 0.06 mm. It is noteworthy that almost all the observed antimicrobial data exhibit statistically significant differences when compared to the tested antibiotics (Chloramphenicol for bacteria and Nystatin for fungi). We believe that according to our results, at the used concentration and the current test conditions (*in vitro*), the APEO has high antimicrobial activity against tested microorganisms and our findings align with previous studies that classify the effectiveness of essential oils via the disc-diffusion test, wherein a diameter of 10 mm or less is deemed indicative of low activity, between >10 and 15 mm suggests moderate activity, and a diameter exceeding 15 mm signifies high activity, with respect to *Pseudomonas aeruginosa, Escherichia coli,*

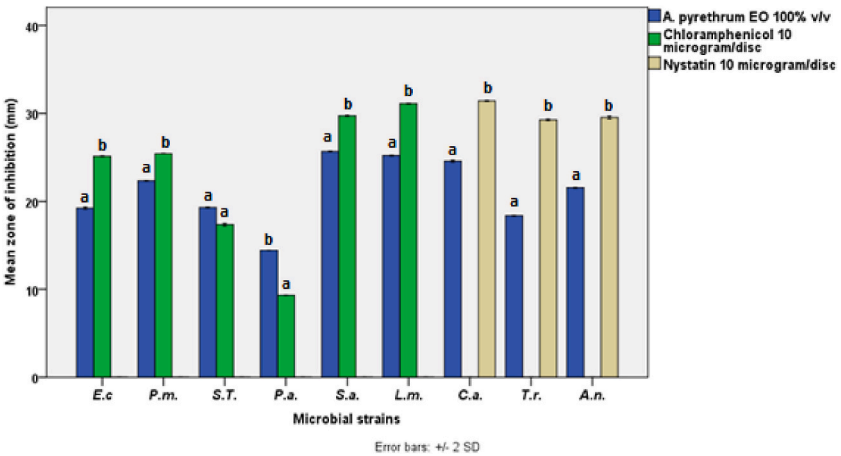

Fig. 2. Assessment of antimicrobial activity of APEO using the disc diffusion method. Data sharing the same letter within the same test indicates no significant difference, as determined by Tukey's multiple range test ($p < 0.05$). E. c.: *Escherichia coli*, P.m.: *Proteus mirabilis*, S.T.: *Salmonella* Typhimurium, P.s.: *Pseudomonas aeruginosa*, S. a.: *Staphylococcus aureus*, L.m.: *Listeria monocytogenes*, C. a.: *Candida albicans*, T. r.: *Trichophyton rubrum*, and A. n.: *Aspergillus niger*.

Klebsiella pneumonia, Staphylococcus aureus, Enterococcus faecalis, Proteus vulgaris, Proteus mirabilis. Streptococcus pyogenes, and *Salmonella typhimurium* [44]. The current results are also in harmony with previous studies on *Anacyclus pyrethrum* EO stated that it has remarkable activity against *Candida albicans* and *Staphylococcus aureus* [41]; *E. coli, Pseudomonas aeruginosa* and *Klebsiella pneumonia* [21]. There is currently no available information regarding the antifungal activity of APEO against the tested dermatophyte fungi, specifically *Trichophyton rubrum*. Finally, it is useful to indicate that disc diffusion offers a simple, cost-effective, and versatile approach for assessing the antibacterial activity of medicinal plants, making it a valuable tool in the field of herbal medicine research [6].

The MIC test (or MFC for fungi) was performed to determine the lowest concentration of essential oil required to inhibit the growth of the microorganisms under examination. Additionally, the MBC assay was executed to ascertain the concentration of the essential oil requisite for the complete eradication of a specific microorganism. Additionally, the MBC assay was executed to ascertain the concentration of the essential oil requisite for the complete eradication of a specific microorganism. The MBC results as well as the MBC/MIC ratios are represented in Table 2.

These findings indicate that APEO exhibits varying MIC values, with a concentration as low as 0.25 % effectively inhibiting visible *in vitro* growth in Gram-positive bacteria, whereas for Gram-negative bacteria, the MIC range spans from 0.5 % to 2.0 %. In the case of fungi, the MIC values vary from 0.5 % to 1.0 %. On the other hand, the MBC or MFC of APEO ranges from 0.25 % to 1.0 % for Gram-positive bacteria and from 0.5 % to 2.0 % for Gram-negative bacteria. Remarkably, the MFC ratio remains consistent at 1.0 % for all tested fungal strains. In the literature, little is known about the MIC, MBC, or MFC testing for APEO. However, the methanolic extract of *A. pyrethrum* was tested against *E. coli*, and the MIC and MBC values were at 800 mg/mL [45]. The methanolic extract of the roots of *Anacyclus pyrethrum* showed inhibitory activity against *S. aureus* and *E. coli* strains, with MICs ranging from 100 to 200 mg/mL, respectively [46]. Given the paucity of available data in published literature pertaining to MIC, MBC, and MFC investigations, more future studies are recommended. Additionally, we calculated the MBC/MIC ratios was ranged between 1.0 and 4.0 for all examined microorganisms, which reflects the bactericidal and fungicidal power of the tested EO. In fact, antimicrobials can be classified as either bactericidal or fungicidal when the ratio of MBC/MIC, as well as the ratio of MFC/MIC, is less than or equal to 4.0. Furthermore, it is considered achievable to reach concentrations of the tested agent sufficient to eradicate 99.9 % of the treated microbes under these circumstances. Moreover, when these ratios exceed 4.0, [3]. Furthermore, it is considered achievable to reach concentrations of the tested agent sufficient to eradicate 99.9 % of the treated microbes at lower ratios of MBC [10]. Moreover, when these ratios exceed 4.0, it may be impractical to administer doses of the tested agent capable of eradicating 99.9 % of the microorganisms, thereby categorizing the agent as bacteriostatic.

3.3. *In silico* ADME analysis

Therefore, *in silico* ADME (Absorption, Distribution, Metabolism, and Excretion) profile of seven compounds namely santolina alcohol, α-pinene, yomogi alcohol, limonene, (E)-β-farnesene, germacrene D and artemisinic alcohol present in *A. pyrethrum* essential oil was predicted using SwissADME server. ADME describe how a drug is absorbed into the body, distributed to tissues, metabolized into different compounds, and excreted from the body. The results obtained from the SwissADME are summarized in Table 3.

The oral bioavailability was evaluated by analyzing the radar plot of six physiochemical properties namely, size, lipophilicity, polarity, solubility, flexibility and saturation. For a compound to be deemed as drug-like, its physiochemical properties should align within the optimal range, represented as the pink region on the radar plot (Fig. 3).

As evident from Fig. 2, all the compounds exhibited favorable values for five physiochemical properties including size, lipophilicity, solubility, flexibility, and saturation, within their respective optimal regions. In the case of α-pinene, limonene, germacrene D and (E)-β-farnesene, no polarity was observed, attributed to the absence of any polar groups in the chemical structure of these compounds.

Subsequently, the boiled-egg plot was generated to predict absorption in the human intestinal tract, permeation through the blood–brain barrier and interaction with P-glycoprotein (Fig. 4). Similarly, the interaction of a drug with P-glycoprotein is pivotal in determining its efflux through biological membranes. Moreover, the absorption of a drug in the human intestinal tract is crucial for the effective delivery into the blood for systemic distribution. Similarly, permeation through the blood-brain barrier is critical for substances to reach the CNS.

In our case, none of the compounds were identified as substrates for P-glycoprotein. All compounds exhibited blood-brain

Table 2
MIC, MBC and MFC values of APEO.

Microorganisms	APEO (% v/v)			Chloramphenicol (μg/mL)			Nystatin (μg/mL)		
	MIC	MBC Or MFC	MBC/MIC Or MFC/MIC	MIC	MBC	MBC/MIC Or MFC/MIC	MIC	MBC	MBC/MIC Or MFC/MIC
E. coli	0.5	0.5	1	0.5	1	2	NT	NT	NT
P. mirabilis	0.5	1	2	0.5	1	2	NT	NT	NT
S. Typhimurium	1	2	2	1	32	32	NT	NT	NT
P. aeruginosa	2	2	1	4	64	16	NT	NT	NT
S. aureus	0.25	1	4	0.25	1	4	NT	NT	NT
L. monocytogenes	0.25	0.25	1	0.25	0.5	2	NT	NT	NT
C. albicans	0.5	1	2	NT	NT	NT	4	4	1
T. rubrum	1	1	1	NT	NT	NT	16	16	1
A. niger	0.5	1	2	NT	NT	NT	16	32	2

Table 3
ADME profile predicted by SwissADME server for the studied compounds.

Compounds	MW	Rotatable bonds	H-bond acceptors	H-bond donors	TPSA Å^2	iLOGP	GI absorption	BBB permeant	P-gp substrate	Lipinski violations
Santolina alcohol	154.25	3	1	1	20.23	2.69	High	Yes	No	0
α-Pinene	136.23	0	0	0	0	2.63	Low	Yes	No	0
Yomogi alcohol	154.25	3	1	1	20.23	2.54	High	Yes	No	0
Limonene	136.23	1	0	0	0	2.72	Low	Yes	No	0
(E)-β-Farnesene	204.35	7	0	0	0	3.86	Low	No	No	0
Germacrene D	204.35	1	0	0	0	3.31	Low	No	No	0
Artimisinic alcohol	220.35	2	1	1	20.23	3.06	High	Yes	No	0

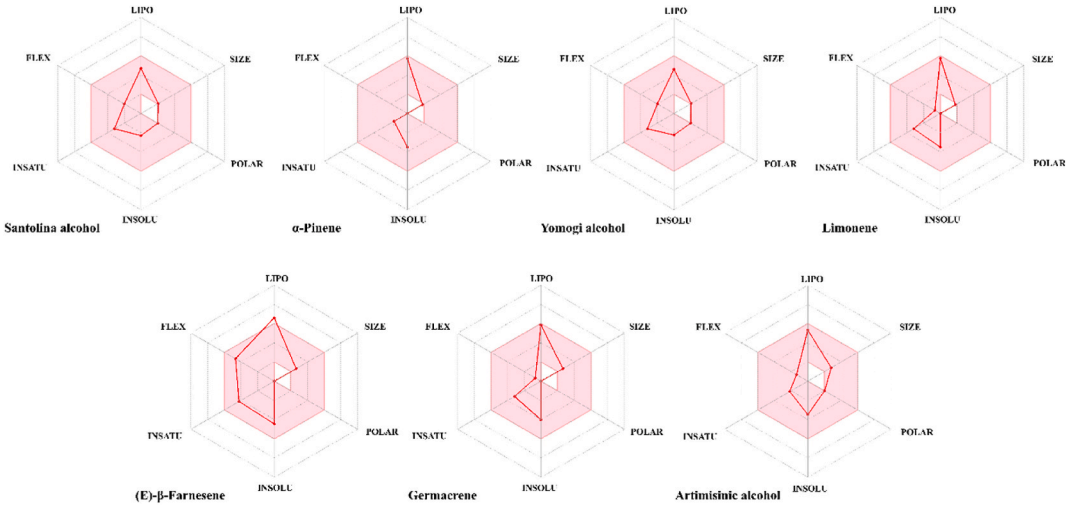

Fig. 3. Bioavailability predictions based on six physiochemical properties for the compounds present in APEO. The properties include size, lipophilicity, solubility, flexibility, saturation, and polarity. The pink region represents the optimal ranges for each property. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

permeation with the exception of germacrene D and (E)-β-farnesene while only santolina alcohol, yomogi alcohol and artemisinic alcohol exhibited passive absorption by the gastrointestinal tract. Additionally, all compounds adhere to Lipinski's rule of five with zero violations and no PAINS alerts.

Similarly, to evaluate the metabolism of selected compounds, the cytochrome p450 (CYP) enzymes inhibition was predicted. In this connection, CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4 inhibition was predicted. None of the compound found to inhibit the aforementioned CYPs, however, α-pinene, limonene, (E)-β-farnesene, germacrene D and artemisinic alcohol found to inhibit CYP2C9. Similarly, the excretion or total clearance was measured by proportionality constant (CLtot) using pkCSM webserver (https://biosig.lab.uq.edu.au/pkcsm/). The CLtot of santolina alcohol, α-pinene, yomogi alcohol, limonene, (E)-β-farnesene, germacrene D and artemisinic alcohol was found to be 0.52, 0.04, 0.47, 0.21, 1.83, 1.4 and 0.1 log (ml/min/kg), respectively. Taken in concert, the ADME profile suggested that the compounds possess desirable properties for further consideration in drug development.

3.4. Molecular docking

DNA gyrase is considered as a potential therapeutic target for the drug design against bacteria due to its pivotal role in bacterial DNA replication and transcription processes by introducing negative supercoiling, which facilitates the separation of DNA strands [47]. In this study, we investigate the molecular interactions between the compounds found in APEO and DNA gyrase B of *Staphylococcus aureus*, to provide a rationalization for the observed antibacterial activity. The results of molecular docking simulation reveal that all the compounds reside well into the binding site of DNA gyrase B enzyme by mediating a number of significant interactions with the binding site residues (Fig. 5).

Insight into the docked pose of santolina alcohol, a network of hydrophobic interactions was observed along with two hydrogen bond contacts. The oxygen of the hydroxyl group mediates two hydrogen bonds with the side chain of Arg84 and Asp57 at a distance of 3.1 Å and 2.3 Å. Similarly, the alkyl group of compounds were observed to mediate hydrophobic interaction with Asn54, Ile86, Ile102 and Leu103. In the binding site of the target enzyme, α-pinene only mediated hydrophobic interactions, with no observed hydrogen bonds. This absence of hydrogen bonding may be attributed to the lack of polar groups in the compound. In this case, Asn54, Glu58,

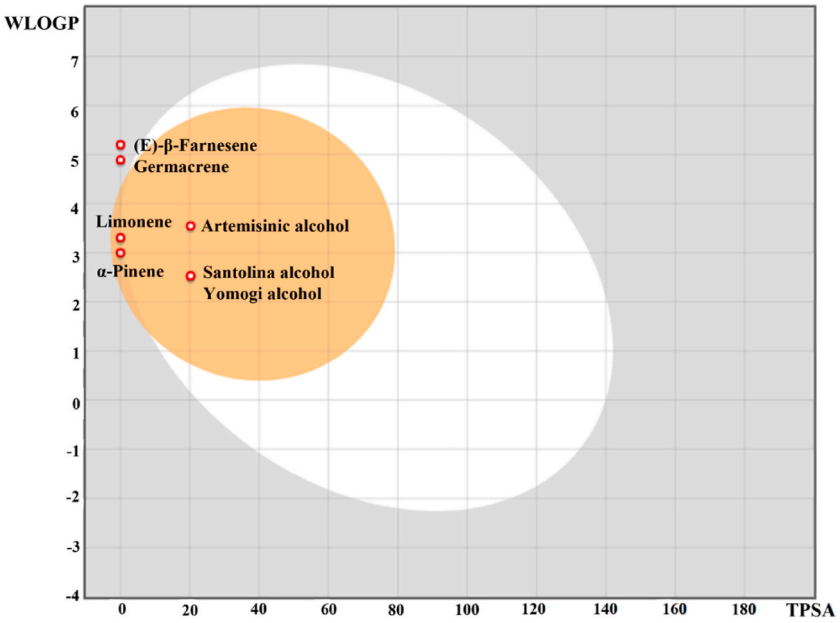

Fig. 4. Boiled egg plot predicting GI absorption, blood-brain barrier permeation, and P-glycoprotein substrate characteristics for the compounds present in APEO. The yellow region indicates the optimal area for blood-brain permeation, the white region represents GI absorption, and the red dots indicate that all compounds are not P-gp substrates. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

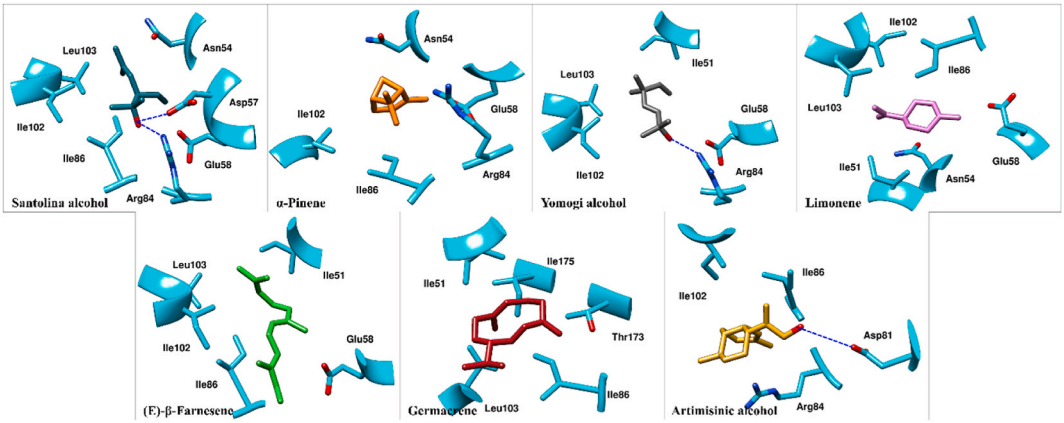

Fig. 5. The intermolecular interactions between the compounds present in APEO and DNA gyrase B of *Staphylococcus aureus* (PDB ID 3G7B). The residues of the enzyme surrounded by the compounds are shown in cyan sticks while the compounds are shown in different color sticks. The blue dotted lines represent the hydrogen bond contacts. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Arg84, and Ile102 contribute to the stabilization of the protein-ligand complex through hydrophobic interactions.

In the case of yomogi alcohol, a strong hydrogen bond was observed along with several hydrophobic interactions. The oxygen of the hydroxyl group mediates a hydrogen bond contact with the side chain of Arg84 at a distance of 2.3 Å. Additionally, hydrophobic interactions were observed with Ile51, Glu58, Ile102, and Leu103. Similarly, in the binding site of the DNA gyrase B, limonene resides firmly by mediating several significant hydrophobic interactions. The cyclohexene ring of the compound engaged in hydrophobic interactions with Ile86 and Asn54, while the alkyl groups formed interactions with Glu58, Ile51, Ile102, and Leu103. Similarly, in the binding site of the DNA gyrase B, (E)-β-farnesene also demonstrated a network of hydrophobic interactions. The residues Ile51, Glu58, Ile86, Ile102, and Leu103 demonstrated a vital role by mediating hydrophobic interactions, thereby contributing to the stabilization of the protein-ligand complex. Similarly, the germacrene also exhibited hydrophobic interactions with the binding site residues of DNA gyrase B. In this case, the residues Ile51, Ile86, Leu103, Thr173, and Ile175 played a crucial role in stabilizing the complex through the formation of hydrophobic interactions.

In case of artemisinic alcohol in the binding site of the enzyme, a hydrogen bond was also observed along with hydrophobic interactions. The oxygen of the hydroxyl group formed a hydrogen bond contact with side chain of Asp81 at a distance of 3.1 Å. While Arg84, Ile86, and Ile102 mediate hydrophobic interactions with the compounds. The docking results inferred that the studied compounds exhibit favorable binding interactions with the DNA gyrase B, which delineate their potential as promising antibacterial candidates for further optimization.

4. Conclusion

APEO is characterized by the presence of oxygenated monoterpene santolina alcohol as a main component. The essential oil showed remarkable antibacterial and antifungal activities. Gram-positive bacteria MIC was found to be 0.25 % v/v, whereas MBC values varied between 0.25 % and 1.0 % v/v. The MBC values of the Gram-negative bacteria were within the range of 0.5 %–2.0 % v/v, and their MIC values ranged from 0.5 % to 2.0 % v/v. The MFC for all examined fungal strains was consistently 1.0 %, whereas the MIC values for the strains varied from 0.5 % to 1.0 % v/v. The results of molecular docking simulation reveal that all the compounds of *A. pyrethrum* essential oil reside well into the binding site of DNA gyrase B enzyme of *Staphylococcus aureus* by mediating a number of significant interactions with the binding site residues. The ADME profile suggested that the major compounds *A. pyrethrum* essential oil possess desirable properties for further consideration in drug development. However, further investigations are strongly required to confirm the potential applications of this essential oil as an antimicrobial agent.

Ethical approval

Not applicable.

Data availability

Data will be made available on request.

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CRediT authorship contribution statement

Aziza El Baz: Writing – original draft, Conceptualization. **Hanae Naceiri Mrabti:** Formal analysis, Data curation. **Naglaa S. Ashmawy:** Supervision, Software, Methodology. **Salman Ali Khan:** Visualization, Software, Methodology. **Emad M. Abdallah:** Supervision, Investigation, Formal analysis. **Samiah Hamad Al-Mijalli:** Methodology, Investigation, Funding acquisition, Formal analysis, Data curation. **Rawaf Alenazy:** Methodology, Investigation, Formal analysis, Data curation. **Fahad M. Alshabrmi:** Validation, Methodology, Investigation, Formal analysis, Data curation. **Abdelhakim Bouyahya:** Writing – review & editing, Validation, Supervision, Conceptualization. **Naoufal El Hachlafi:** Writing – original draft, Investigation, Formal analysis. **Chrismawan Ardianto:** Writing – review & editing, Supervision, Conceptualization. **Farida ifadotunnikmah:** Writing – review & editing, Supervision, Formal analysis. **Fouzia Hmimid:** Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Samiah Hamad Al-Mijalli reports was provided by Princess Nourah bint Abdulrahman University. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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