European Journal of Medicinal Plants

33(7): 28-40, 2022; Article no.EJMP.88899
ISSN: 2231-0894, NLM ID: 101583475

Evaluation of Physiochemical Properties, Thermal Behavior and Phytopharmaceutical Potential of *Citrus aurantium's* Essential Oil

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2022/v33i730479

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/88899

Original Research Article

Received 01 May 2022
Accepted 04 July 2022
Published 08 July 2022

ABSTRACT

The study investigates the therapeutic potential of the *Citrus aurantium var. amara* essential oil extracted from the blossoms of the bitter orange plant by examining its chemical composition, thermal stability, and potency against infectious disease-causing pathogens. Initially, the volatile components of the essential oil were evaluated by obtaining a chromatographic fingerprint using HPTLC and FTIR spectrum identification. Furthermore, a thermal profile of the essential oil was obtained using the thermogravimetric-differential thermal analysis and differential scanning calorimetric analysis. A predetermined set of antibiotic-resistant microorganisms were used to examine the antibacterial activity of the essential oil. Lastly, its anti-inflammatory activity was assessed using the albumin denaturation assay. The research concluded that the *Citrus aurantium var. Amara* essential oil exhibits potential therapeutic characteristics which can be further explored through in vivo studies.

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Keywords: *Antibacterial; anti-inflammatory; antibiotic-resistance; citrus aurantium var. amara; differential scanning calorimetry; essential oil; fourier-transform infrared spectrometry; high-performance thin layer chromatography; thermogravimetric-differential thermal analysis.*

ABBREVIATIONS

EO : Essential Oil
CaEO : Citrus aurantium var. amara essential oil
HPTLC : High-Performance Thin Layer Chromatography
FTIR : Fourier-transform infrared spectrometry
TGA-DTA : Thermogravimetric-Differential Thermal Analysis
DSC : Differential Scanning Calorimetry
CRA : Carbapenem-resistant Acinetobacter spp.
CRP : Carbapenem-resistant Pseudomonas aeruginosa
CRE : Carbapenem-resistant Escherichia coli
CRK : Carbapenem-resistant Klebsiella pneumonia
ESBL : Extended-spectrum beta-lactamase Escherichia coli
VRE : Vancomycin-resistant Escherichia coli
QRS : Quinolone-resistant Salmonella
MRSA : Methicillin-resistant Staphylococcus aureus
ERS : Erythromycin-resistant Streptococci
BSA : Bovine serum albumin
DMSO : Dimethyl Sulfoxide Solution

1. INTRODUCTION

With the widespread exploitation of antibiotics, the growth and spread of antibiotic-resistant bacteria have been increasing perennially. Thus, antibiotic resistance is among the top most crucial public health issues of the twenty-first century [1].

With an increase in the prevalence of antibiotic-resistant microorganisms, fewer antimicrobial therapies are available to treat diseases caused by these pathogens. This calls for an urgent need for alternative strategies to subdue the spread of antibiotic-resistant bacteria, currently being explored by research groups around the globe [2]. Considering their low toxicity, pharmacological actions, and economic feasibility, plant medicinal characteristics have turned a few heads in the advancement of science. Phytochemicals and their impact on human health have become key specific for such investigations [3].

Essential oils (EOs), also known as volatile oils, encompass a significant group of antimicrobial components (complex combinations of secondary metabolites) derived from plants (leaves, buds, fruits, flowers, herbs, twigs, bark, wood, roots, and seeds). The antiseptic, antibacterial, antiviral, antioxidant, antiparasitic, antifungal, and insecticidal properties of essential oils have been documented. Consequently, essential oils can be a pertinent tool in the fight against bacterial resistance [4]. They are known for their extensive use in wellness culture to attain various benefits, as flavouring agents and aroma disseminators.

Usually, EOs are obtained by steam distillation or cold pressing a specific part of a plant. The appropriate method is chosen according to the concentration of the required constituent. Cold pressing is preferred when the raw material has a low concentration of highly volatile components such as hydrocarbon and terpenic derivatives [5]. Essential oils comprise hundreds of biostructures made up of several distinct compounds, each with its own set of qualities (antiseptic, antibacterial, immune- stimulatory, decongestants, etc.). These biostructures operate collectively, which explains the adaptability and broad-spectrum of EOs. The relevance (beneficial or detrimental) of essential oils can be assessed by understanding the concentration and qualities of biostructures.

The complex biostructures of essential oils derived from distinct plants are composed of various fully or partially saturated molecules representing chemical classes such as terpenoids, ketones, aldehydes, esters, etc. The heterogeneity of essential oils from similar

species could be governed by increased chemical complexity, topographical and climatic conditions, and the uniqueness of production and/or purifying technologies. Thus, making it difficult to establish the identity of their biostructures [6].

Citrus, a genus belonging to the Rutaceae family, comprehends a wide range of species with diverse sizes and shapes, including lemons, limes, oranges, mandarins, citrons, and grapefruits. Traditionally, Citrus plants were related to herbal medicine in Asian countries, especially Japan, China, and Korea. *Citrus aurantium var. amara*., also known as sour orange, bitter orange, Seville orange, or bigarade, is a five metre tall evergreen tree renowned for its fragrant white blossoms [7]. They are assumed to have antibacterial, anti-inflammatory, and antioxidant properties. Such bioactivities are exhibited due to the presence of components such as phenolics, flavonoids, essential oil, and vitamins [8]. Monoterpene hydrocarbons make up a large portion of citrus essential oils (70-95%), with minor amounts of sesquiterpene hydrocarbons, oxygenated derivatives, and aromatic hydrocarbons. Limonene, α-pinene, β-pinene, β-myrcene, and linalool are the primary constituents of citrus oil. Although these fruits are usually used in the preparation of desserts, their essential oils have great commercial significance. Citrus fruit essential oils are the most extensively used essential oils in the world and are derived as by-products of citrus processing. The principal components of these essential oils have acquired approval in the food industry on account of their safety and tolerance by an array of food products [9]. Therefore, amassing an in- depth knowledge of the chemical compositions and bioactivity of these organic compounds can be highly beneficial [10].

The current study aimed to investigate the chemical composition of the *Citrus aurantium var. amara* essential oil (CaEO) using HPTLC and FTIR analyses, thermal properties using differential scanning calorimetry (DSC) and thermogravimetric- differential thermal analysis (TG-DTA). The antibacterial characteristics and anti- inflammatory potential of CaEO were investigated using a wide range of antibiotic resistant microorganisms by the broth microdilution method and the albumin denaturation assay, respectively.

2. MATERIALS AND METHODS

2.1 Procurement of Essential Oil

For extraction of essential oil, *Citrus aurantium var. amara* blooms were obtained from Solangapalayam, Erode, Tamil Nadu, India (11°13'00.4"N 77°50'19.2"E). Approximately, 350g of the dried and crushed blossoms were homogenized in 1L of Distilled water, as described by the Liang Z,et al. [11]. Further, the oil was extracted by hydro distillation method using a Clevenger apparatus for 3 hours in a 2 L round-bottom flask [12-13]. Residual moisture present in the extracted oil were eliminated by using anhydrous sodium sulphate. The oil obtained was stored at 4℃ in dark until further experimental analysis.

2.2 HPTLC

Preparation of essential oil sample:

The concentrated extracts of the CaEO were reconstituted with 20 mL of methanol and refrigerated at 4℃ for further testing [14].

Software and instrumentation: For the establishment of the chromatographic profile, a High-Performance Thin Layer Chromatography (HPTLC) analysis was performed. The current analysis referred to the methods deduced by Akbar et al. with slight modifications [15]. Using the Linomat V applicator, approximately 2 µL of the prepared EO sample was applied on an aluminium plate pre-coated with Silica gel 60 F254 of 100 mm thickness. HPTLC software (vision CATS, CAMAG, Muttenz, Switzerland) was used for data processing and analysis.

Derivatization:

Ensuing the development of TLC plates using Toluene: Ethyl acetate, 9.7:0.3 v/v solvent system, they were derivatized using vanillin-sulphuric followed by heat treatment at 120℃ for 3 minutes. Finally, the plates were observed under UV 254 nm and UV 366 nm.

2.3 FTIR

The Carry 630 Fourier transform infrared spectrometer was used to undergo the functional group analysis of the CaEO sample. The apparatus was initially pre-heated and stabilised. A drop of the EO sample was placed on a clean pellet of Potassium chloride (KCl) upon which

another pellet was sandwiched to allow the formation of a thin film between the two plates, ensuring the absence of air bubbles. The KCl pellets were gently rotated to make the oil create a uniform liquid membrane. Before attaching and inserting the KCl pellets into the infrared spectrometer sample holders, the infrared spectrometer was calibrated to an absorbance with a resolution of 1 cm^{-1} and a 45-time repetition scan before gently rotating. The infrared absorption spectra of the oil samples were collected in the spectral region ranging from 4000 to 650 cm^{-1} with a resolution of 8 cm.

2.4 TG-DTA

The thermo gravimetric analysis device model "DSC SDT Q600" was used to make the measurements and analyses of the acquired CaEO sample considering the work done by Hazra et al. as a reference [16]. The EO sample was placed in an aluminium crucible under an inert nitrogen gas atmosphere with a flow rate of about 300 mL/min. The crucible containing the EO weighed 23.01 mg and measurements were noted from 25℃ to 500℃ with a heat influx rate of about 20℃/min.

2.5 DSC

To determine the differential scanning calorimetry (DSC) profile of the CaEO oil sample, model "DSCQ20" from TA Instruments, a division of Waters Corporation, was used. A sample weight of 10 mg was placed in aluminium crucibles under a contained flow of nitrogen gas (40 mL/min). A dynamic scan was performed at a heating rate of 20℃/min over a temperature range of 25 to 400℃.

2.6 Antibacterial

The antibacterial activity of the CaEO sample was carried out on a 96 well microplate in triplicates using the micro broth dilution method. The method devised by Cazella et al.[17] was referred to with some additional changes. The test organisms against which the antibacterial activity of the EO was to be examined consisted of CRA (Carbapenem-resistant *Acinetobacter spp.*), CRP (Carbapenem-resistant *Pseudomonas aeruginosa*), CRE (Carbapenem-resistant *Escherichia coli*), CRK (Carbapenem-resistant *Klebsiella pneumoniae*), ESBL (Extended-spectrum beta-lactamase *Escherichia coli*), VRE (Vancomycin-resistant *Escherichia coli*), QRS (Quinolone-resistant *Salmonella*), MRSA (Methicillin-resistant *Staphylococcus aureus*) and ERS (Erythromycin-resistant *Streptococci*). Initially, the EO was diluted in a 5 % dimethyl sulfoxide solution (DMSO) with 0.1 % polysorbate-80 (1 mg/mL) to make up a stock solution of 1000 mg/mL. Later, the stock concentration was serially 2-fold diluted and introduced to each test organism grown in Luria-Bertini (LB) broth with cell viability of 1.0 x 10^4 CFU/well. Additionally, to assess bacterial density, 9.95 ml of 1% H2SO4 and 0.05 ml of 1% BaCl2 were mixed to form the McFarland number 0.5 standard [18]. More over,The test organisms were subsequently added to all the wells, with the exception of wells in column 11, which contained broth and neat DMSO as a negative control. By introducing the broth and test organism in the wells of column 12, the ability of the media to support the growth of the test organism, as positive control, was assessed. After that, the plates were covered with a sterile sealer and kept at 37°C for 24 hours. For bacterial growth assessment, p-iodonitrotera zolium chloride (INT, Sigma) [0.2 mg/ml] was introduced to each well and incubated at 37°C for 30 minutes in a rotary agitator (180 rpm). Bacterial growth was detected by the development of a pink-red colouration, whereas growth inhibition was indicated by the maintenance of a clear coloration. The MIC was defined as the lowest concentration that showed colour change [19].

2.7 Anti-Inflammatory

The anti-inflammatory potential of the EO was evaluated as implemented by Foe et al. [20] using the albumin denaturation assay, with few modifications. The reaction mixture included a range of diluted CaEO oil concentrations and a 30% bovine serum albumin (BSA) fraction prepared in dimethyl sulfoxide and sterile distilled water (negative control). After 20 minutes of incubation at 37ºC, the test tubes were heated for 20 minutes at 60^{o}C. A UV-Visible spectrophotometer (ThermoFisher Scientific) was used to measure the absorbance of these solutions at a wavelength of 660 nm. The percentage inhibition of precipitation (i.e. albumin denaturation) was calculated using the formula:

$$Percentage\ inhibition = \frac{Absorbance\ (control) - Absorbance\ (sample)}{Absorbance\ (control)} x\ 100z$$

3. RESULTS AND DISCUSSION

3.1 HPTLC Analysis

The assessment and quantification of phytochemicals using high-performance thin-

layer chromatography (HPTLC) is a simple, fast, accurate, and reliable approach. Its efficiency over other chromatographic methods has contributed to its global acceptance. The use of chromatographic fingerprints to examine and confirm the quality of plant species with active chemical constituents is a feasible procedure in traditional medicine. Besides assessing specific markers of interest, chromatographic fingerprints of essential oils can also detect the presence of other components [21]. Such an HPTLC technique was devised to detect the various components of *Citrus aurantium var. amara* (Neroli) essential oil.

The study focuses on improving the HPTLC fingerprint profiles of components found in *Citrus aurantium var. amara* essential oil. Bands of blue, purple, and light brown were generated. The densitogram revealed two distinct peaks [22].

Vanillin-sulfuric acid, a universal derivatizing agent for natural products, was used in this study. It is considerably used in the detection of terpenoids/steroids as they (monoterpenes, triterpenes, and steroids) appear in distinct hues. Under white light, monoterpenes, triterpenes, and steroids appear as dim grey, blue or red-violet (purple), and grey spots, respectively. Colour distinction extends from mild grey to blue, violet to rose-red, and green, aiding in the identification of specific components [23]. Figs. (1) and (2), show the HPTLC peak and fingerprint under UV 254 and 366 nm, respectively [24].

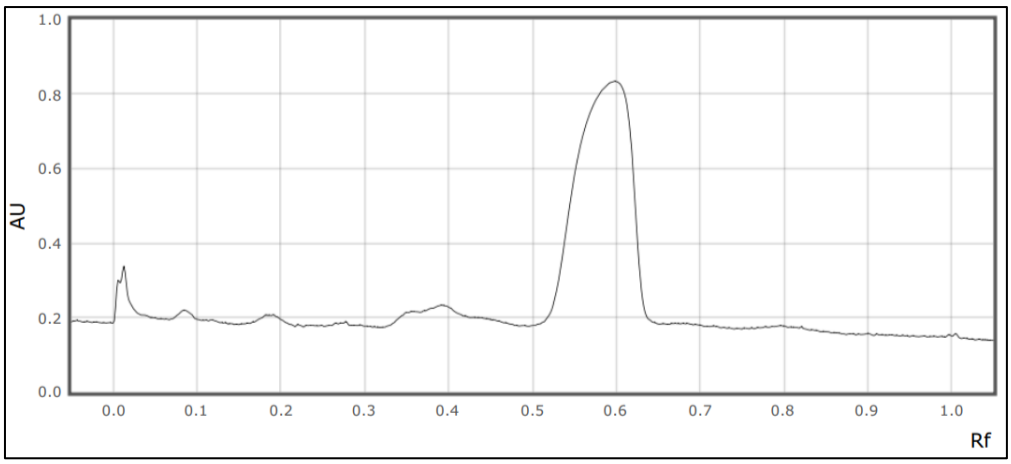

Fig. 1. HPTLC densitogram profile of the *Citrus aurantium var. amara* essential oil

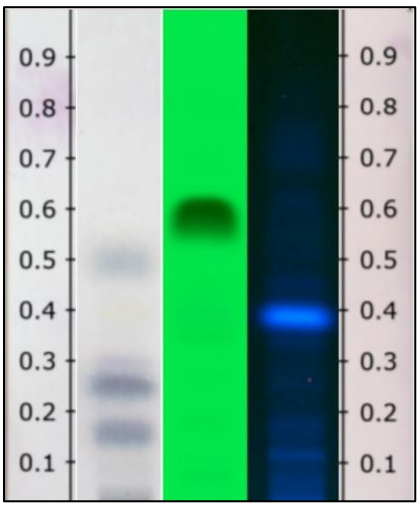

Fig. 2. HPTLC fingerprint of *Citrus aurantium var. amara* essential oil
a. After derivatizaton using Vanillin sulphuric acid; b. After development at UV 254 nm, and c. After development at UV 366 nm, respectively

3.2 FTIR Analysis

Fourier-transform infrared (FTIR) spectroscopy is a significant tool for determining the chemical composition of biomolecules. It provides information on the biological substances present in a given sample by outlining various functional groups and chemical bonds. Spectral examination of specimens from the same species enables the assessment of variations in their chemical profiles. As a result, FTIR spectroscopy promises to be a useful instrument for investigating distinctions in the quantities of major organic components between species as well as specimens [25].

Essential oils are refined solutions of volatile components with complex FTIR spectra arising due to the overlapping of individual component spectra and the mixing of multiple vibrational modes [26]. The vibrational frequencies, in wavenumbers, of the sample spectrum produced from the FTIR spectrometer were compared with those of an IR correlation chart [27]. A mid-range FTIR spectrum was plotted with wavenumbers ranging from 4,000 to 650 cm^{-1} as shown in Fig. (3).

Citrus aurantium var. amara's spectral graph depicted various characteristic functional groups generally found in essential oils. The peak at 3529.8 represents intermolecular bonded O-H stretch alcohol. Peaks at 2959.5, 2926.0, and 2862.6 delineate C-H stretch alkane, whereas peaks at 2109.7 and 1986.7 show the occurrence of C≡C terminal alkyne (monosubstituted) and N=C=S stretch isothiocyanate respectively. Carboxylic acid, C=C-C aromatic ring stretch, and Methyl C-H asymmetrical/symmetrical bend were observed at peaks 1722.0, 1580.4, and 1461.1, respectively. O-H bending carboxylic acid compound, aromatic ethers**,** alkyl-substituted ether C-O stretch, cyclic ethers-large rings-C-O stretch, C-O stretching primary alcohol, and C=C bending were observed at peaks 1397.1, 1267.3, 1121.9, 1073.5, 1039.9, and 991.5. Peaks at 834.9, 741.7 and 704.5 sequentially represented a trisubstituted medium C=C bending alkene, a monosubstituted C-H bending and a benzene derivative [28].

3.3 Thermogravimetric Analysis

Thermogravimetry (TG) is a domain of thermal analysis that primarily deals with the measurement of sample mass change determined as a function of changing temperature or isothermally as a function of time in a specified atmosphere of nitrogen, oxygen, argon, helium, air, or vacuum. Thermogravimetric analysis (TGA) is a method of determining the weight of a substance as a function of time or temperature under controlled heating or cooling conditions.

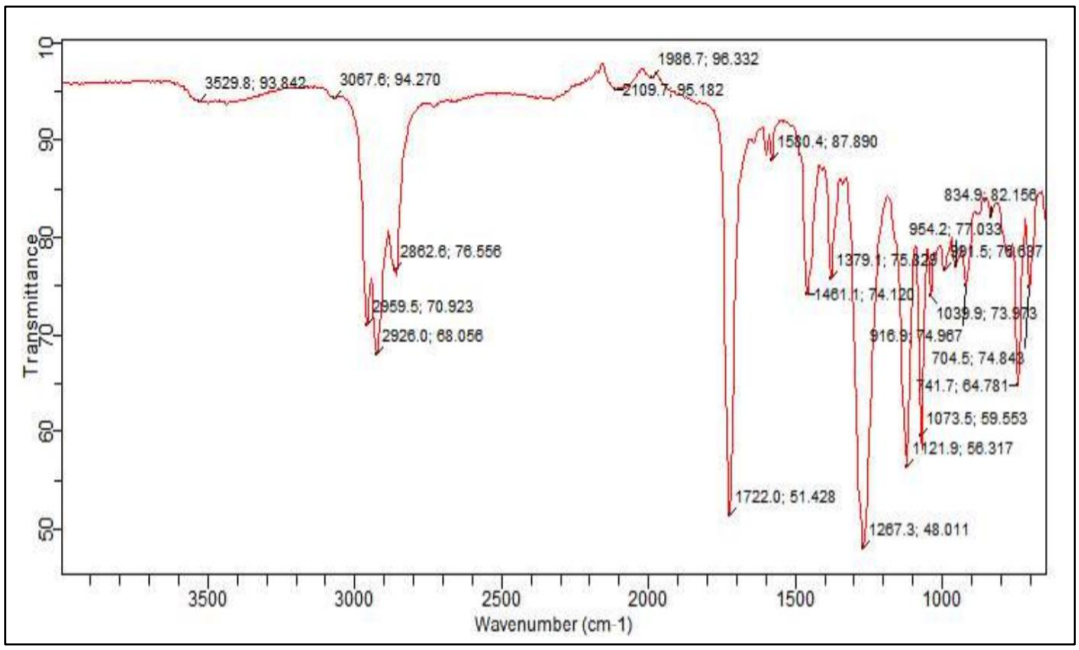

Fig. 3. FTIR spectra of *Citrus aurantium* var. amara essential oil ranging from wavenumbers 4,000-650 cm-1 versus transmittance

Table 1. FTIR spectra table of the CaEO

Peak	Functional group	Transmittance
3529.8	O-H stretch alcohol	93.842
2959.5	C-H stretch alkane	70.923
2926.0	C-H stretch alkane	68.065
2862.6	C-H stretch alkane	76.556
2109.7	C≡C Terminal alkyne (monosubstituted)	95.182
1986.7	N=C=S stretch isothiocyanate	96.332
1722.0	Carboxylic acid	51.428
1580.4	C=C-C Aromatic ring stretch	87.890
1461.1	Methyl C-H asymmetrical/symmetrical Bend	74.120
1397.1	O-H bending carboxylic acid	75.829
1267.3	Aromatic ethers, aryl -O stretch	48.011
1121.9	Alkyl-substituted ether, C-O stretch	56.317
1073.5	Cyclic ethers, large rings, C-O stretch	59.553
1039.9	S=O stretching sulfoxide	73.973
991.5	C=C bending alkene	76.687
954.2	C=C bending disubstituted (trans)	77.033
834.9	C=C bending alkene trisubstituted	82.156
741.7	C-H bending monosubstituted	64.781
704.5	benzene derivative	74.848

This method arose to meet the dire need for a technique capable of efficiently controlling the temperature as well as producing a temperature versus time programme within specific prerequisites. Chemical reactions, such as combustion, involve mass loss, while physical changes include phase change, such as melting, which can't be considered a loss. The effect of heating rate, gas flow rate, experimental atmosphere, nature of, sample and particle size are identified as state-of-art biomass characterization for future research [29].

This thermogravimetric curve (TGA) of CaEO, with the increase in temperature, demonstrates the results of breakdown reactions or mass loss due to high temperature. The 23.01 mg EO sample considered for the study was subjected to a heat flow of up to 20°C/min, leading to a loss of 99.74 % as shown in Fig. (4). A loss in the mass of the EO sample was first observed at 67-68°C. The curve kept extending up to approximately 355°C after which it stabilized at a lower point. These results denote that the selected essential oil is highly stable. The overall analysis reached completion at around 500°C. The leftover residues showed no exothermic characteristics. High thermal degradation temperatures correspond with highly stable and thermally resistant components.

The exothermic peak on the differential thermal analysis (DTA) curve was seen at around 345°C, indicating a release of sample energy due to component breakdown. Thus, the thermal stability of *Citrus aurantium var. amara* essential oil was validated by DTA.

3.4 Calorimetry Profile

Differential scanning calorimetry (DSC) helps determine a molecule's heat flow as a function of time and temperature. The process is carried out by measuring the difference in the energy delivered to the sample and the reference pan as a function of temperature. Physical and chemical events can be observed by altering the energy of the sample as a function of temperature. Endothermic effects are produced by dehydration, phase transitions, reduction, and decomposition reactions typically, whereas oxidations, crystallization, and some decomposition reactions give rise to exothermic effects [30].

Thermal analyses of the 10 mg CaEO coated particles revealed a drastic increase in endothermic heat flow from 30°C to 125°C, after which a gradual transition into exothermic heat flow was observed. As seen in Fig. (5), the curves corresponding to the EO do not show a sharp peak but rather demonstrate the volatility of its components from 146.49°C to 222.08°C. This suggests that the phase change materials (PCMs) possess high thermal energy storage. The initial EO mass loss, as observed in Fig. (5), from 30°C to 125°C was due to the loss of moisture and other volatile material in the PCMs followed by the exothermic transition of the

remnant material. The majority of mass loss was observed on approaching 222.08°C and continued up to 278.93°C. Subsequently, the shift from 300°C to 400°C resulted in total exothermic combustion. This conveyed that the EO components were moderate to highly stable.

3.5 Antibacterial

This investigation demonstrated the antibacterial activity of CaEO against selected antibiotic-resistant pathogens. As shown in Table (2) and Fig. (6), the MIC of CaEO against the selected bacteria ranged from 50 to 200 µg/mL. Among the Gram-positive species, CaEO showed the strongest activity against Erythromycin-resistant *Streptococci* (ERS) [31].

Similar to the findings obtained from the studies conducted by Hsouna et al. [32], CaEO showed antibacterial activity against both, Gram-negative and Gram-positive bacteria with the former group more vulnerable than the latter. The antibacterial activity

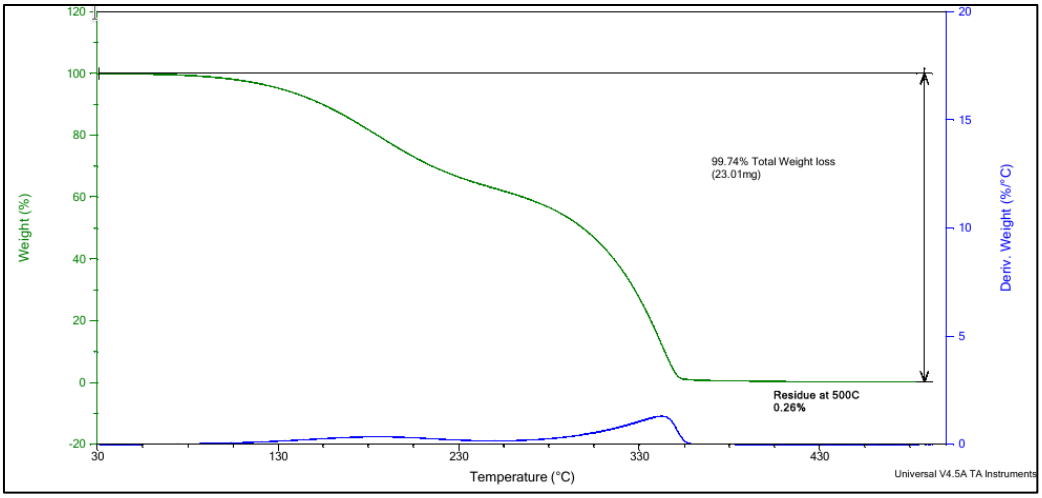

Fig. 4. Thermogravimetric analysis of *Citrus aurantium var. amara* essential oil

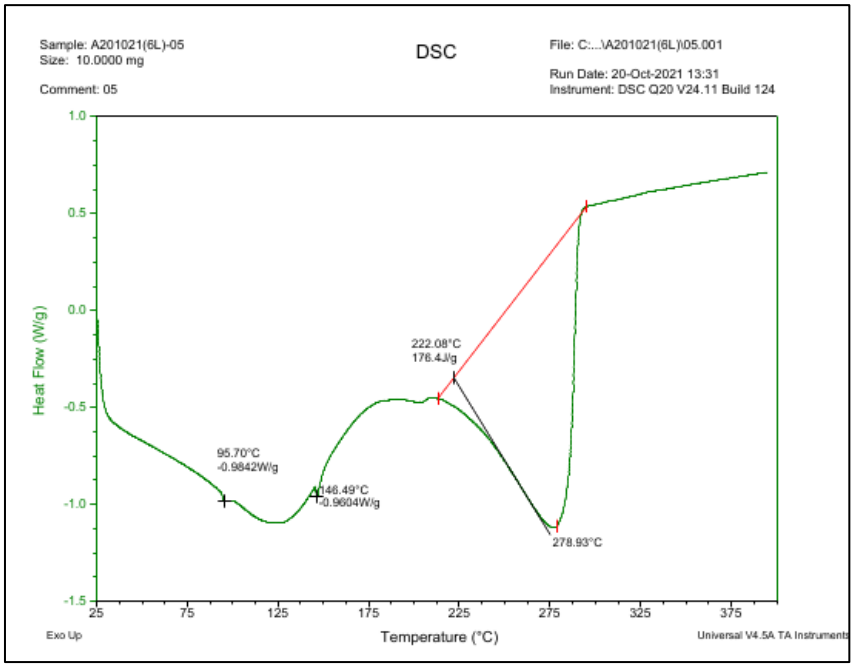

Fig. 5. Differential scanning calorimetry (DSC) profile analysis of *Citrus aurantium var. amara* essential oil

of CaEO primarily depends on its concentration and the bacterial strain examined by it. Observational discrepancies could arise considering the greater complexity of Gram-negative cell envelopes in comparison to its counterpart. CaEO's potential to weaken the permeability barrier of cell membrane structures eventually leading to impaired chemiosmotic regulation is the most plausible cause of its fatal impact. Overall, CaEO showed moderate sensitivity against the Gram-negative test organisms. It showed the highest potency against Quinolone-resistant *Salmonella* (QRS) and Extended-Spectrum beta-lactamase *Escherichia coli* (ESBL) with MIC values of 50 and 62.5 µg/mL, respectively. Thus, significantly inhibiting growth at low concentrations.

A thorough literature search brought to light the activity of major (β-pinene, linalool, α-terpineol, β-myrcene, D-limonene, β-ocimene, nerol, nerolidol, linalyl acetate, etc…) and minor (hexadecanoic acid ester, benzoic acid methyl ester etc) components of CaEO against bacterial pathogens. This bioactivity can be accounted for by considering the synergistic activity of both components against harmful bacteria [31]. Based on these findings, CaEO could be used as a natural remedy against drug resistant microorganisms.

3.6 Anti-inflammatory

Inflammation is a natural protective response to tissue injury or infection. It aids the body to resist intruders (pathogens and non-self-cells) and discards dead or damaged host cells. Certain plants are widely acknowledged as an important source of novel chemical compounds with medicinal value. Owing to their low cost and reduced side effects, the use of herbal medicine and natural products has shot up in recent years [33]. Essential oils are widely utilized as an alternative to traditional anti-inflammatory medications. Substantial research has accentuated the anti-inflammatory action of essential oils from plants belonging to the Citrus family [34]. Thus, justifying the anti-inflammatory properties of the CaEO.

This study employed the denaturation of egg albumin methodology to assess the anti-inflammatory properties of CaEO. On completion of the experiment, it was inferred that CaEO protected the albumin

Table 2. Minimal Inhibition Concentration (MIC) of *Citrus aurantium var. amara* essential oil against drug-resistant bacteria

Sr. No.	Test Organisms	MIC (µg/mL)
1.	CRA(Carbapenem-resistant *Acinetobacter spp.*)	125
2.	CRP (Carbapenem-resistant *Pseudomonas aeruginosa*)	200
3.	CRE (Carbapenem-resistant *Escherichia coli*)	125
4.	CRK (Carbapenem-resistant *Klebsiella pneumoniae*)	125
5.	ESBL (Extended-spectrum beta-lactamase *Escherichia coli*)	62.5
6.	VRE (Vancomycin-resistant *Escherichia coli*)	100
7.	QRS (Quinolone-resistant *Salmonella*)	50
8.	MRSA (Methicillin-resistant *Staphylococcus aureus*)	125
9.	ERS (Erythromycin-resistant *Streptococci*)	100

Table 3. Anti-inflammatory activity of *Citrus aurantium var. amara* essential oil at varying concentrations (10-100 µg/mL)

Concentration of EO (µg/mL)	O.D. at 660 nm	% Inhibition
10	1.562	15
20	1.329	28
30	0.829	55
40	0.738	60
50	0.426	76
60	0.430	76.6
70	0.335	81.89
80	0.232	87.45
90	0.125	93
100	0.121	93.45
Control (EW)	1.85	0

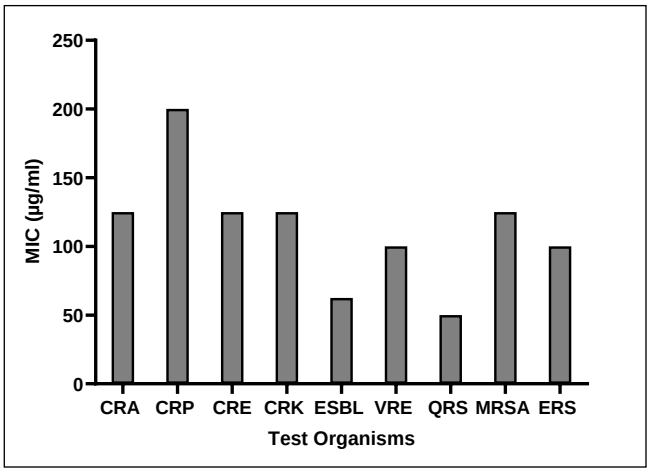

Fig. 6. Graphical representation of the MIC of Citrus aurantium var. amara essential oil against drug-resistant bacteria.CRA (Carbapenem-resistant Acinetobacter spp), CRP (Carbapenem-resistant Pseudomonas aeruginosa), CRE(Carbapenem-resistant Escherichia coli), CRK (Carbapenem-resistant Klebsiella pneumoniae), ESBL (Extended spectrum beta-lactamase Escherichia coli), VRE (Vancomycin-resistant Escherichia coli), QRS (Quinolone-resistant Salmonella), MRSA (Methicillin-resistant Staphylococcus aureus) and ERS (Erythromycin-resistant Streptococci)

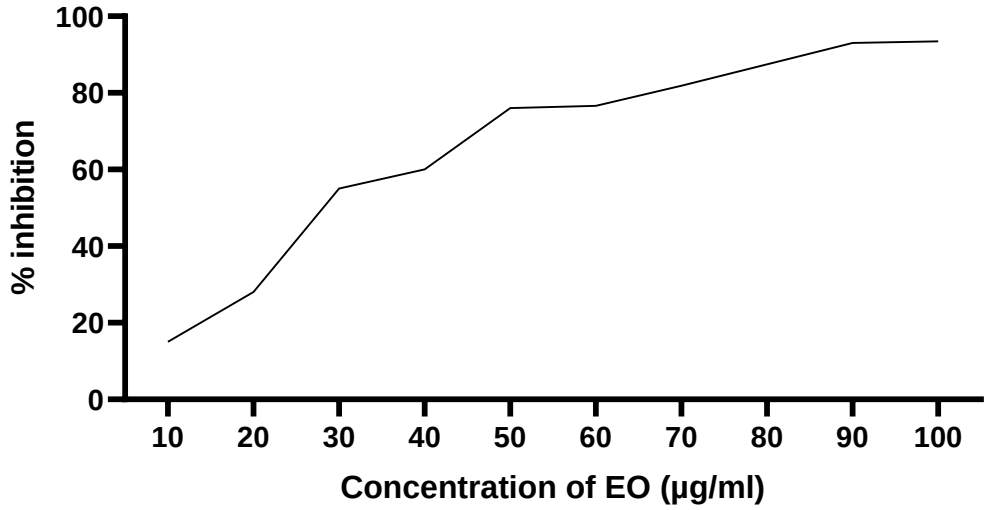

Fig. 7. Anti-inflammatory activity of *Citrus aurantium var.* amara essential oil against percentage Inhibition

protein from denaturation caused by heat. An increase in CaEO concentration was observed to be directly proportional to albumin protein protection, indicating the ability of CaEO to inhibit protein denaturation as shown in Table (3) and Fig. (7). Protein denaturation in inflammatory diseases such as rheumatoid arthritis, cancer, and diabetes, promotes the formation of autoantigens. This can be controlled using CaEO as an inhibitor of protein denaturation.

4. CONCLUSION

Given the various adverse effects linked with the continuous use of synthetic drugs, this study strived to evaluate and promote the usage of easily accessible natural compounds against infectious diseases to overcome antibiotic resistance. On examining the distinct characteristics of the essential oil extracted from *Citrus aurantium var. amara* (Neroli) blossoms,

the research findings were successful in demonstrating the phyto pharmaceutical potential of CaEO. Following the extraction of the essential oil, % yield was found to be 3.18 and Densitometry- HPTLC and FTIR analyses were used to determine the chemical composition of the EO. CaEO's thermal stability was supported using thermal analysis. The EO showed antimicrobial activity against several test antibiotic-resistant bacteria with low MIC values against Gram-negative organisms like Quinolone- resistant *Salmonella* (QRS) and Extended-Spectrum *Escherichia coli* (ESBL). Besides this, it also possessed anti-inflammatory properties. Thus, in conclusion, CaEO can be employed as a natural antibacterial agent to diagnose a range of infectious diseases. Consequently, the bitter orange plant could be a novel source for isolating antibacterial compounds that could be used in the preparation of functional foods and medicines. To clarify the mode(s) of action of the plant chemicals, more research is in fact required to pinpoint the precise active substances. Moreover, discovery of the active phytochemicals responsible for the antibacterial activity, further phytochemical characterization and scientific evaluation of these plants should be conducted. The EO can be further explored and validated in terms of its toxicity and *In vivo* potency. This would further advocate its safety concerning plant-drug interactions and optimal dose.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Wang CH, Hsieh YH, Powers ZM, Kao CY. Defeating Antibiotic-Resistant Bacteria: Exploring Alternative Therapies for a Post-Antibiotic Era. Int J Mol Sci 2020, Vol 21, Page 1061. 2020;21(3):1061. DOI:10.3390/IJMS21031061
2. Vivas R, Barbosa AAT, Dolabela SS, Jain S. Multidrug-Resistant Bacteria and Alternative Methods to Control Them: An Overview. Microb Drug Resist. 2019; 25(6):890-908. DOI:10.1089/MDR.2018.0319/ASSET/IMAGES/LARGE/FIGURE1.JPEG
3. Chouhan S, Sharma K, Guleria S. Antimicrobial Activity of Some Essential Oils—Present Status and Future Perspectives. Medicines. 2017;4(3):58. DOI:10.3390/MEDICINES4030058
4. Magi G, Marini E, Facinelli B. Antimicrobial activity of essential oils and carvacrol, and synergy of carvacrol and erythromycin, against clinical, erythromycin-resistant Group A Streptococci. Front Microbiol. 2015;6(MAR):165. DOI:10.3389/FMICB.2015.00165/ABSTRACT
5. Tanhaeian A, Hadi Sekhavati M, Moghaddam M. Antimicrobial activity of some plant essential oils and an antimicrobial-peptide against some clinically isolated pathogens. Chem Biol Technol Agric. 2020;7:13. DOI:10.1186/s40538-020-00181-9
6. Samfira I, Rodino S, Petrache P, Cristina RT, Butu M, Butnariu M. Characterization and identity confirmation of essential oils by mid infrared absorption spectrophotometry ♣. Dig J Nanomater Biostructures. 10(2):557-566.
7. Maksoud S, Abdel-Massih RM, Rajha HN, et al. Citrus aurantium L. Active Constituents, Biological Effects and Extraction Methods. An Updated Review. Mol 2021, Vol 26, Page 5832. 2021; 26(19):5832. DOI:10.3390/MOLECULES26195832
8. Hsouna A Ben, Hamdi N, Halima N Ben, Abdelkafi S. Characterization of essential oil from Citrus aurantium L. flowers: antimicrobial and antioxidant activities. J Oleo Sci. 2013;62(10):763-772. DOI:10.5650/JOS.62.763
9. Rowshan V, Najafian S. Journal of Essential Oil Bearing Plants Changes of Peel Essential Oil Composition of Citrus aurantium L. During Fruit Maturation in Iran Changes of Peel Essential Oil Composition of Citrus aurantium L. During Fruit Maturation in Iran. Published online; 2015. DOI:10.1080/0972060X.2014.977564
10. Iseppi R, Mariani M, Condò C, Sabia C, Messi P. Essential Oils: A Natural Weapon against Antibiotic-Resistant Bacteria Responsible for Nosocomial Infections. Antibiot 2021, Vol 10, Page 417. 2021; 10(4):417. DOI:10.3390/ANTIBIOTICS10040417
11. Liang Z, Shang XB, Su J, et al. Alternative Extraction Methods of Essential Oil From the Flowers of Citrus aurantium L. Var Daidai Tanaka: Evaluation of Oil Quality and Sedative-Hypnotic Activity: Available:https://doi.org/101177/1934578X211004061. 2021;16(3).

DOI:10.1177/1934578X211004061
12. Moradi S, Fazlali A, Hamedi H. Microwave-Assisted Hydro-Distillation of Essential Oil from Rosemary: Comparison with Traditional Distillation. Vol 10.
13. Al-Harrasi A, Al-Saidi S. Phytochemical Analysis of the Essential Oil from Botanically Certified Oleogum Resin of Boswellia sacra (Omani Luban). Molecules. 2008;13(9):2181. DOI:10.3390/MOLECULES13092181
14. Anerao J, Jha V, Desai N. Optimization of DNA Extraction Methods from Garcinia species for ISSR-PCR, RAPD-PCR and DNA Barcoding. Asian J Biotechnol. 2016;9(1):35-42. DOI:10.3923/AJBKR.2017.35.42
15. Akbar N, Katiyar A, Vilas G, Srivastava S, Saxena BK. Isolation and Chromatographic Fingerprint (HPTLC) Analysis of Resinoid from Refuse/Waste material of Nagarmotha. Available:http://dx.doi.org/101080/22297928201110648245. 2013;1(5-6):402-405. DOI:10.1080/22297928.2011.10648245
16. Hazra A, Alexander K, Dollimore D, Riga A. Characterization of some essential oils and their key components. Thermoanalytical techniques. J Therm Anal Calorim. 2004;75(1):317-330. DOI:10.1023/B:JTAN.0000017352.86803.6D
17. Cazella LN, Glamoclija J, Soković M, et al. Antimicrobial activity of essential oil of baccharis dracunculifolia DC (Asteraceae) aerial parts at flowering period. Front Plant Sci. 2019;10:27. DOI:10.3389/FPLS.2019.00027/BIBTEX
18. De Zoysa MHN, Rathnayake H, Hewawasam RP, Wijayaratne WMDGB. Determination of in Vitro Antimicrobial Activity of Five Sri Lankan Medicinal Plants against Selected Human Pathogenic Bacteria. Int J Microbiol. 2019;2019. DOI:10.1155/2019/7431439
19. Njeru SN, Obonyo MA, Nyambati SO, Ngari SM. Antimicrobial and cytotoxicity properties of the crude extracts and fractions of Premna resinosa (Hochst.) Schauer (Compositae): Kenyan traditional medicinal plant. BMC Complement Altern Med. 2015;15(1):1-9. DOI:10.1186/S12906-015-0811-4/TABLES/9
20. Ndoye Foe FMC, Tchinang TFK, Nyegue AM, et al. Chemical composition, in vitro antioxidant and anti-inflammatory properties of essential oils of four dietary and medicinal plants from Cameroon. BMC Complement Altern Med. 2016;16(1). DOI:10.1186/S12906-016-1096-Y
21. Alqarni MH, Foudah AI, Alam A, Salkini MA, Alam P, Yusufoglu HS. Novel HPTLC-densitometric method for concurrent quantification of linalool and thymol in essential oils. Arab J Chem. 2021; 14(2):102916. DOI:10.1016/J.ARABJC.2020.102916
22. Phytochemical profile and antioxidant activity of the essential oil from Blumea eriantha DC. Accessed May 6, 2022. Available:https://www.researchgate.net/publication/267035961_Phytochemical_profile_and_antioxidant_activity_of_the_essential_oil_from_Blumea_eriantha_DC
23. Romero Rocamora C, Ramasamy K, Meng Lim S, Majeed ABA, Agatonovic-Kustrin S. HPTLC based approach for bioassay-guided evaluation of antidiabetic and neuroprotective effects of eight essential oils of the Lamiaceae family plants. J Pharm Biomed Anal. 2020;178. DOI:10.1016/J.JPBA.2019.112909
24. Senguttuvan J, Subramaniam P. HPTLC Fingerprints of Various Secondary Metabolites in the Traditional Medicinal Herb Hypochaeris radicata L. J Bot. 2016;2016. DOI:10.1155/2016/5429625
25. Durak T, Depciuch J. Effect of plant sample preparation and measuring methods on ATR-FTIR spectra results. Environ Exp Bot. 2020;169:103915. DOI:10.1016/J.ENVEXPBOT.2019.103915
26. Agatonovic-Kustrin S, Ristivojevic P, Gegechkori V, Litvinova TM, Morton DW. Essential oil quality and purity evaluation via ft-ir spectroscopy and pattern recognition techniques. Appl Sci. 2020; 10(20):1-12. DOI:10.3390/APP10207294
27. Catauro M, Bollino F, Tranquillo E, et al. Chemical analysis and anti-proliferative activity of Campania Thymus Vulgaris essential oil. J Essent Oil Res. 2017; 29(6):461-470. DOI:10.1080/10412905.2017.1351405
28. Nandiyanto ABD, Oktiani R, Ragadhita R. How to read and interpret ftir spectroscope of organic material. Indones J Sci Technol. 2019;4(1):97-118. DOI:10.17509/IJOST.V4I1.15806
29. Olatunji OO, Akinlabi SA, Mashinini MP, Fatoba SO, Ajayi OO. Thermo-gravimetric

characterization of biomass properties: A review. IOP Conf Ser Mater Sci Eng. 2018;423(1). DOI:10.1088/1757-899X/423/1/012175

30. Komesu A, Martins Martinez PF, Lunelli BH, Oliveira J, Wolf MacIel MR, MacIel Filho R. Study of lactic acid thermal behavior using thermoanalytical techniques. J Chem. 2017;2017. DOI:10.1155/2017/4149592
31. Değirmenci H, Erkurt H. Relationship between volatile components, antimicrobial and antioxidant properties of the essential oil, hydrosol and extracts of Citrus aurantium L. flowers. J Infect Public Health. 2020;13(1):58-67. DOI:10.1016/J.JIPH.2019.06.017
32. Hsouna A Ben, Hamdi N, Halima N Ben, Abdelkafi S. Characterization of essential oil from Citrus aurantium L. flowers: Antimicrobial and antioxidant activities. J Oleo Sci. 2013;62(10):763-772. DOI:10.5650/JOS.62.763
33. Dharmadeva S, Galgamuwa LS, Prasadinie C, Kumarasinghe N. In vitro anti-inflammatory activity of Ficus racemosa L. bark using albumin denaturation method. Ayu. 2018;39(4):239. DOI:10.4103/AYU.AYU_27_18
34. Shen CY, Jiang JG, Zhu W, Ou-Yang Q. Anti-inflammatory effect of essential oil from citrus aurantium L. var. amara Engl. J Agric Food Chem. 2017;65(39):8586-8594. DOI:10.1021/ACS.JAFC.7B02586

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/88899