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ARTICLES

Constituents and Antibacterial Activity of Essential Oil of *Nyctanthes arbor-tristis* Flower

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Abstract—In this study, the composition and antibacterial activity of essential oil from the flower of *Nyctanthes arbor-tristis* were determined. The extraction was carried out utilizing the microwave-assisted extraction technique with a short extraction time of 60 min, yielding 0.342% (w/w). Separation and characterization were performed by gas chromatography-mass spectrometric analysis, identifying a total of 34 constituents, including major compounds: phenyl ethyl alcohol (26.1%), eucarvone (18.7%), furfural (10.7%), benzaldehyde (4.8%), phytol (4.7%), and methyl hexadecanoate (3.1%). In the next step, an agar disk diffusion assay was performed to assess the antimicrobial activity of the oil against four different bacterial strains, namely *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli*, and *Pseudomonas aeruginosa*, resulting in zones of inhibition measuring 9, 12, 11, and 9 mm, respectively. However, the broth macrodilution method was carried out against *Staphylococcus aureus* and *Klebsiella pneumoniae*, revealing minimum inhibitory concentrations of 2.5 and 5 mg/mL, respectively.

Keywords: *Nyctanthes arbor-tristis* flower, phenylethyl alcohol, eucarvone, antibacterial activity, microwave-assisted extraction

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Nyctanthes arbor-tristis is an important plant of the Oleaceae family [1], known as the Olive family, which comprises 24 genera and 615 species distributed all over the world, excluding the Arctic region. Native to Southeast Asia, it is largely found in Pakistan, India, Bangladesh, and Nepal. This plant is typically cultivated in gardens from July to October, due to its sweet jasmine-like fragrance [2]. The *Nyctanthes* genus includes *Nyctanthes aculeata* (native only to Thailand) and *Nyctanthes arbor-tristis,* both of which contain similar pharmacological compounds such as triterpenoids, flavonol glycosides, loganins, phenylpropanoid glucoside, and iridoid glycosides [3]. *Nyctanthes arbor-tristis*, commonly known as "Har singhar", is a shrub with highly fragrant flowers with a corolla of six tube-shaped petals resembling jasmine. It is an important part of Unani and Indian Ayurveda. Fresh leaves are used for malaria treatment, while dried-flower extracts are taken by females to address infertility issues [4]. Pharmacologically, *Nyctanthes arbor-tristis* has been reported to possess antimicrobial, antifungal, antioxidant, anti-inflammatory, anticancer, antiviral, cytotoxic, antiproliferative, and anti-depressant properties [5–7].

Various techniques are employed for the extraction of essential oils such as hydrodistillation, steam distillation, supercritical fluid extraction, expression, headspace solid-phase microextraction, solvent extraction, and microwave-assisted extraction (**MAE**). However, MAE has gained significant attention in research and development due to its shorter extraction time and efficiency comparable to traditional methods [8]. In many cases, the time for essential oil extraction can be reduced to 30–45 min [9, 10], compared to other common traditional techniques especially hydrodistillation and steam distillation, which may take up to 5 h [2, 11]. Additionally, MAE offers advantages such as low solvent utilization, minimal waste production, a reduction in the release of toxic solvents into the environment, and decreased human exposure to solvents [12]. To overcome the drawbacks associated with both modern and traditional extraction tech-

niques such as cost, time, thermolability, or amount of solvent, MAE is considered a significant approach in the field of green technology [12].

Previously, various extracts of *Nyctanthes arbor-tristis* have been studied [13–16]. Studies on its flower essential oil (**FEO**) have been reported using traditional hydro- and steam distillation methods [2, 11, 17], whereas the leaf and bark were studied using hydrodistillation [18, 19]. However, there has been no previous study on the microwave-assisted extraction of FEO. Additionally, there was a need for systematic characterization of its essential oil constituents, including additional confirmation through retention indices. Furthermore, there was a knowledge gap regarding the antimicrobial activity of FEO. Considering the significant potential that essential oils possess against various ordinary and multidrug-resistant pathogenic bacteria [20–22], and the above-mentioned knowledge gap, the current study was conducted using the advanced MAE technique followed by systematic compound identification and evaluation of the antibacterial activity of its flower essential oil.

EXPERIMENTAL

Plant material and microwave extraction of essential oil. Fresh flowers were obtained from Lahore (Pakistan), and the plant was identified as *Nyctanthes arbor-tristis* by Dr. Abdul Rehman Khan Niazi. A voucher specimen, LAH#29621, was submitted to the herbarium of the University of the Punjab, Lahore (Pakistan). 96 g of fresh flowers were subjected to extraction in a distillation assembly using a modified domestic microwave from Orient, Pakistan (model number OM46SS, output voltage 1000 W, and output frequency 2450 MHz) for 60 min. The power level was adjusted to 60% to control and regulate a constant temperature. No water was added for the extraction, and two extractions were performed. The essential oil was further extracted using the solvent extraction technique with dichloromethane as the collecting solvent. The sample was then kept at −10°C for gas chromatography-mass spectrometric analysis. For antibacterial analysis, the solvent was later evaporated below 40°C, and the essential oil was used for the antibacterial assay.

Gas chromatography-mass spectrometry. An Agilent gas chromatography-mass spectrometry (**GC-MS**) instrument (5977 A series) with a fused silica capillary column DB-5ms (inner diameter 180 μm, film thickness 0.18 μm) was used. Helium was employed at a flow rate of 1 mL/min. The temperature ramp mode was used, starting at 40°C and held for 4 min, and then increased to 280°C at the rate of 10°C/min and was again held for 2 min. The mass-to-charge ratio (*m/z*) range employed was 35 to 450. Compound identification was conducted using MSD ChemStation with NIST library 2011 as a reference. Moreover, saturated alkanes purchased from Sigma Aldrich (C_7–C_{30}) were analyzed under the same conditions for dual confirmation. Retention index values were calculated and compared with standard values in Adams 2007 literature or NIST online data [23, 24].

Antibacterial assay. Four multidrug-resistant (**MDR**) bacterial strains, comprising gram-negative *Escherichia coli* (5964-LF), *Pseudomonas aeruginosa* (5994-NLF), *Klebsiella pneumoniae* (599-BLF), and gram-positive *Staphylococcus aureus* (613-B), were collected from the Microbiology Lab of Sheikh Zayed Hospital, Lahore, Pakistan. The antibacterial potential of the essential oil was determined using the agar disk diffusion method. To prepare volumes of known concentration, the essential oil was dissolved in dimethyl sulfoxide (**DMSO**) at concentrations of 54 mg/0.5 mL DMSO (used for the macro broth dilution method) and 329 mg/mL of DMSO (used for the agar disk diffusion method). Luria-Bertani agar medium was poured into sterilized Petri dishes, and four disks of 6 mm diameter were placed individually on the surface of each media plate. A sample volume of 20 μL (equivalent to 6.58 mg) was applied to three disks, while 20 μL of DMSO was applied to the fourth disk as the negative control, using a micropipette. After incubating the plates for 24 h at 37°C, the inhibition zones were measured in millimeters.

Macrobroth dilution assay. Minimum inhibitory concentrations (**MIC**s) and minimum bactericidal concentrations (**MBC**s) of the essential oil against two different bacterial strains were also determined using a 48-well plate against *Klebsiella* spp. and *Staphylococcus aureus*. Inoculums were prepared by dissolving the strains in sterilized 0.85% saline solution to make 0.5 McFarland standard. The turbidity of the inoculum was adjusted to a bacterial count equal to 10^5 CFU/mL using sterilized broth. A volume of 185 μL of the oil sample containing 10 mg of oil was added to the 1st and 2nd wells for each strain. First, 815 μL of inoculum was added to the first two wells of each strain, and 500 μL of inoculum was added from the 3rd to the 8th well by transferring 500 μL solution from the 2nd well to the 8th well, and the sample solution from the last well was discarded. In this way, a concentration range of 10 to 0.078 mg/mL was prepared. Finally, 500 μL of inoculum was added to each well from the 2nd to the 8th for each strain. Negative controls (broth only) and positive controls (inoculum and broth) were set in the last two rows. The multiwell plate was then incubated for 24 h at 37°C in an oven. MIC was determined after 24 h by evaluating the turbidity of the wells, and MBC was calculated after incubating for 3 days under the same conditions.

RESULTS AND DISCUSSION

In this study, a pale yellow-colored essential oil was obtained from the flower essential oil of *Nyctanthes arbor-tristis.* MAE provided an extraction time of only

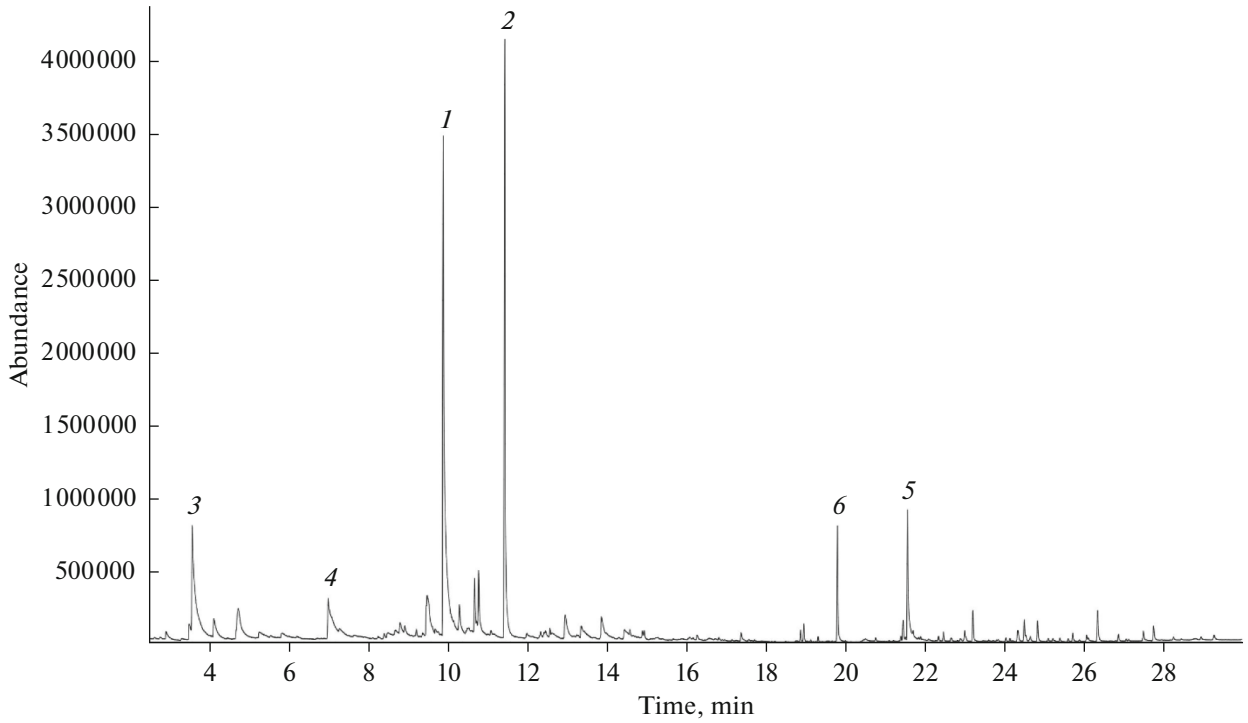

Fig. 1. Total ion chromatogram of *Nyctanthes arbor-tristis* flower essential oil showing major compounds: (*1*) phenyl ethyl alcohol, (*2*) eucarvone, (*3*) furfural, (*4*) benzaldehyde, (*5*) phytol, (*6*) methyl hexadecanoate.

60 min, however, in previous studies conducted on FEO, the extraction times were approximately 5 h, indicating a significant reduction in time in the current study. Additionally, the extraction yield in this study (0.342%, w/w) was higher compared to previous studies, where it ranged from 0.06 to 0.295% (w/w) of the fresh flower [2, 11]. A total of 34 components were identified in this study, with phenyl ethyl alcohol (26.1%), eucarvone (18.7%), furfural (10.7%), benzaldehyde (4.8%), phytol (4.7%), and methyl hexadecanoate (3.1%) being the most abundant. These findings are presented in Table 1, and the total ion chromatogram is presented in Fig. 1.

In terms of constituents, FEO of *Nyctanthes arbor-tristis* obtained by steam distillation contained benzyl benzoate (17.3%), benzyl acetate (16.1%), hedione (11.7%), *n*-hexyl cinnamaldehyde (7.4%), phenyl ethanol (4.6%), and benzyl alcohol (4.6%) in abundance [17]. When hydrodistillation was applied to petal and corolla tubes, the primary constituents were 2-methyloctadecane (17.6%) and 3,7,11,15-tetramethyl-2-hexadecene-1-ol (40.3%) [2], however, in another study, hydrodistillation of fresh flowers provided phytol (32.2%) and methyl palmitate (14.7%) as major constituents. Comparing to these previous studies, out of a total of 34 identified constituents in this study, the identical compounds were: benzaldehyde, β-phorone, epoxylinalol, linalool oxide, safranal, eucarvone, hexahydrofarnesyl acetone, methyl hexadecanoate, methyl elaidate, phytol, muscalure, heptacosane, and tricosane whereas the remaining 21 constituents were not present in any of the essential oils reported earlier.

Studies on essential oils extracted from other parts of *Nyctanthes arbor-tristis* using hydrodistillation for 4 h revealed low yields of 0.002 and 0.005%, respectively, and presented 20 compounds in the bark compared to a total of 26 compounds in the leaf. Both oils contained comparable proportions of hexadecanoic acid (34.3 and 26.4%, respectively) and octadecanoic acid (3.9 and 6.2%, respectively). However, both leaf and bark oils contained numerous components that were present in one but absent in the other, such as (E)-phytol, (3Z)-hexenyl benzoate, linalool, β-eudesmol, and other eudesmol isomers, etc. [18], where few of these compounds such as phenylethyl alcohol, methyl palmitate, phytol, and *p*-vinylguaiacol were present in FEO in the current study. Another study on leaf essential oil using hydrodistillation for 4 h identified 22 compounds with eugenol (88.2%) being the most dominant compound [19], which was largely different in terms of quality and quantity of compound from the other study on its leaf [18] as well as from the current study where phytol was the only compound that was identical. Hence, comparison with the above-mentioned studies demonstrates that the extraction time in the study has been significantly reduced to 1 h along with an improved yield.

Various other extracts were also analyzed. GC-MS analysis of leaf ethanol extract identified a total of five

Table 1. Volatile components of flower of *Nyctanthes arbor-tristis*

no.	Retention time, min	Compound	Chemical formula	RI_{Lit}	RI_c	Relative abundance, %
1	3.598	Furfural	$C_5H_4O_2$	837	836	10.7
2	4.140	*trans*-2-Hexenal	$C_6H_{10}O$	858	858	1.3
3	5.289	4-Cyclopentene-1,3-dione	$C_5H_4O_2$	902	911	0.3
4	5.857	Ethanone, 1-(2-furanyl)	$C_6H_6O_2$	927	927	0.2
5	7.014	Benzaldehyde	C_7H_6O	976	976	4.8
6	8.428	β-Phorone	$C_9H_{14}O$	1047	1044	0.2
7	8.826	Benzyl alcohol	C_7H_8O	1068	1065	0.6
8	9.512	Benzoic acid, methyl ester	$C_8H_{10}O$	1105	1105	4.0
9	9.910	Phenylethyl alcohol	$C_8H_{10}O$	1130	1130	26.1
10	10.320	Oxopholone	$C_9H_{12}O_2$	1155	1152	1.5
11	10.699	Epoxylinalol	$C_{10}H_{18}O_2$	1179	1173	1.6
12	10.801	Linalool oxide	$C_{10}H_{18}O_2$	1185	1184	2.1
13	11.116	Safranal	$C_{10}H_{14}O$	1205	1205	0.1
14	11.461	Eucarvone	$C_{10}H_{14}O$	1229	1223	18.7
15	12.977	*p*-Vinylguaiacol	$C_9H_{10}O_2$	1335	1334	1.9
16	13.379	Methyl anthranilate	$C_8H_9NO_2$	1364	1363	0.7
17	13.891	Cinnamic acid	$C_{10}H_{10}O_2$	1402	1402	1.5
18	14.922	Dehydro-β-ionone	$C_{13}H_{18}O$	1484	1485	0.2
19	14.964	β-Ionon-5,6-epoxide	$C_{13}H_{20}O_2$	1487	1488	0.3
20	18.979	Hexahydrofarnesyl acetone	$C_{18}H_{36}O$	1843	1843	0.5
21	19.824	Methyl hexadecanoate	$C_{17}H_{34}O_2$	1927	1927	3.1
22	21.417	Methyl linoleate	$C_{19}H_{34}O_2$	2093	2092	t
23	21.474	Methyl elaidate	$C_{19}H_{36}O_2$	2099	2089	0.6
24	21.587	Phytol	$C_{20}H_{40}O$	2111	2111	4.7
25	22.365	Docosane	$C_{22}H_{46}$	2198	2200	0.1
26	22.490	Phytyl acetate	$C_{22}H_{42}O_2$	2213	2218	0.2
27	23.024	Muscalure	$C_{23}H_{46}$	2275	2274	0.4
28	23.229	Tricosane	$C_{23}H_{48}$	2299	2300	0.9
29	24.859	Pentacosane	$C_{25}H_{52}$	2499	2500	0.6
30	26.368	Heptacosane	$C_{27}H_{56}$	2698	2700	1.1
31	27.149	Supraene	$C_{30}H_{50}$	2808	2808	t
32	27.517	2-Methyloctacosane	$C_{29}H_{60}$	2861	2861	0.3
33	27.775	Nonacosane	$C_{29}H_{60}$	2898	2900	0.5
34	29.299	Triacontane	$C_{30}H_{62}$	2997	3000	0.2

RI_{Lit}—retention indices obtained from NIST library, Adams literature, and online databases; RI_c—retention indices calculated relative to C_7–C_{30} authentic standards under the same conditions as the essential oil; t—trace (<0.1%).

Table 2. Classification of different essential oil constituents of *Nyctanthes arbor-tristis* flower

Class of volatile constituent	Serial number of constituents from table 1	Percentage, %
Monoterpenes	11, 13, 14, 19	20.7
Diterpenes	24, 26	5.0
Aldehydes	1, 2, 5	16.9
Ketones	3, 4, 6, 10, 20	2.7
Fatty acid	21, 22, 23	3.8
Alkanes	25, 28, 29, 30, 32, 33, 34	3.8
Alcohols and phenols	7, 9, 15, 16	29.3
Others	8, 12, 31, 17, 18, 27	8.2
Total		90.4

compounds, with palmitic acid and *cis*-9-hexadecenal in abundance [13]. The ethyl acetate extract of the leaf revealed the presence of 11 compounds in the extract, with geranyl geraniol, palmitic acid, and benzoic acid as major compounds. Out of these, only cinnamic acid and phytol were present in the study on FEO [15].

The current phytochemical profile of flower essential oil also enabled to identify the industrially important, potentially therapeutic, and biologically active agents such as furfural, *trans*-2-hexenal, 4-cyclopentene-1,3-dione, benzaldehyde, benzyl alcohol, benzoic acid, methyl ester, benzene ethanol, oxopholone, and epoxylinalol. Furfural is used in adhesives, fungicides, fertilizers, and flavoring compounds, as well as in the production of furfuryl alcohol and other applications such as chemical, medicinal, and flavoring intermediates. It is a crucial curing agent in different respiratory, cardiovascular, pancreatic, and colon illnesses due to its antioxidant and anti-inflammatory properties [25]. *trans*-2-Hexenal has been reported as a significant essential oil component. 4-Cyclopentene-1,3-dione acts as an active antitumor agent [26, 27], while benzaldehyde has antifungal properties [28, 29]. Benzyl alcohol is used in anti-parasitic medications. Benzoic acid, methyl ester, and benzene ethanol have been reported to possess antibacterial and antiviral activity, respectively [30–32]. Oxopholone is a well-known aromatic component [33], while epoxylinalol demonstrates phytotoxic potential [34].

The data on the characterization of various major compounds present in flower essential oil are as follows:

(1) Furfural: RT 3.59 min: *m/z* 96.10 (100, M^+), 67.10 (7), 39.10 (42). Both mass spectral matching similarity index (**SI**) 940 and retention index values (Lit = 837, Cal = 836) verify the compound identification;

(2) Benzaldehyde: RT 7.01 min: *m/z* 106.10 (100, M^+), 105.1 (99), 77.10 (93), 73.10 (3), 63.10 (6), 57.10 (62), 53.10 (41), 43.10 (31), 39.10 (15). Both mass spectral matching SI 920 and retention index values (Lit = 976, Cal = 976) verify the compound identification;

(3) Phenylethyl alcohol: RT 9.910 min: *m/z* 122.10 (28, M^+), 92.10 (57), 91.10 (100), 65.10 (15). Both mass spectral matching SI 940 and retention index values (Lit = 1130, Cal = 1130) verify the compound identification;

(4) Eucarvone: RT 11.46 min: *m/z* 150.10 (74, M^+), 135.10 (30), 122.10 (13), 107.10 (100), 91.10 (45), 79.10 (25), 66.10 (34), 43.10 (3), 39.10 (22). Both mass spectral matching SI 860 and retention index values (Lit = 1229, Cal = 1223) verify the compound identification;

(5) Methyl hexadecanoate: RT 19.824 min: *m/z* 270.30 (10, M^+), 239.30 (7), 227.20 (12), 143.10 (17), 129.10 (7), 87.10 (69), 74.10 (100), 55.10 (21), 43.10 (24). Both mass spectral matching SI 990 and retention index values (Lit = 1927, Cal = 1927) verify the compound identification;

(6) Phytol: RT 21.58 min: *m/z* 296.20 (0, M+), 123.20 (28), 122.10 (2), 111.10 (9), 95.10 (16), 81.10 (26), 71.10 (100), 57.10 (27), 43.10 (27). Both mass spectral matching SI 980 and retention index values (Lit = 2111, Cal = 2111) verify the compound identification.

The classification of essential oil components is provided in Table 2. Alcohols and phenols, such as benzyl alcohol, phenyl ethyl alcohol, *p*-vinylguaiacol, and methyl anthranilate, respectively, dominate the flower oil with 29.3%. This is followed by monoterpenes at 20.7%, including compounds such as epoxylinalol, safranal, eucarvone, β-ionon-5,6-epoxide, and aldehyde like furfural, *trans*-2-hexenal, benzaldehyde at 16.9%. Aromatic alcohols and phenolic compounds are known for their lethal action against bacteria, especially gram-negative ones, as they inhibit bacterial growth by denaturing and disrupting cell membranes and proteins [35]. Monoterpenes are important components of plant essential oils by contributing to the aroma and flavor of the plant. Natural and synthetic monoterpenes have been reported to exhibit antibacterial, antifungal, anticancer, anti-inflammatory, and antioxidant activities [36]. Aldehydes also act as important antibacterial agents by destroying bacterial outer cell membranes.

Table 3. Antibacterial activity of *Nyctanthes arbor-tristis* flower oil against bacterial strains

no.	Name of bacteria	Type of resistance	Zones of inhibition, mm (20 μL, 6.58 mg)	Negative control DMSO	MIC, mg/mL	MBC, mg/mL
1	*Klebsiella pneumoniae*	MDR	12	0	5	5
2	*Staphylococcus aureus*	MDR	09	0	2.5	5
3	*Escherichia coli*	MDR	11	0	–	–
4	*Pseudomonas aeruginosa*	MDR	09	0	–	–

Antibacterial activity. Oils are hydrophobic, and because of their permeability and proton motive force on cell membranes, they have a lethal effect on pathogenic bacteria; thus, membrane permeability is an important factor in this regard. Although the plant is traditionally an important herbal remedy for many ailments in Asian countries like Pakistan, India, and Nepal, the flower of this plant has been scarcely employed for its antibacterial activity compared to others. This study documented the positive antibacterial potential results of *Nyctanthes arbor-tristis* flower essential oil in Table 3 against multidrug-resistant bacterial strains. Activity (agar disk diffusion method) is shown in the following order: *Klebsiella pneumoniae* (12 mm) > *Escherichia coli* (11 mm) > *Pseudomonas aeruginosa* = *Staphylococcus aureus* (9 mm) when subjected to 6.58 mg of drug concentration. MIC and MBC against two strains, multidrug resistant *Klebsiella pneumoniae* (MIC and MBC = 5 mg/mL), and multidrug-resistant *Staphylococcus aureus* (MIC and MBC = 2.5 and 5.0 mg/mL, respectively), were also obtained.

Previous studies have shown that methanolic and ethanolic extracts of *Nyctanthes arbor-tristis* leaves have antibacterial activity against *Staphylococcus aureus* (MIC = 62.5 and 72.5 mg/mL, respectively), *Escherichia coli* (MIC = 75.0 and 31.0 mg/mL, respectively), but not against *Pseudomonas aeruginosa* [4]. In this study, MIC obtained by essential oil was much lower in the case of multidrug-resistant *S. aureus*. Moreover, ether and methanolic extract of *Nyctanthes arbor-tristis* fruit showed some activity against *E. coli* (Zone of inhibition = 5 mm) [37, 38]. A study on the essential oil of its leaf exhibited maximum antibacterial potential against *K. pneumoniae* and *P. aeruginosa* with an inhibition zone of 23.8 and 26.3 mm at a concentration of 1000 μg/mL, respectively [19]. In another study, petroleum ether, diethyl ether, and ethyl acetate fractions of its flower were evaluated against *Bacillus subtilis, Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* where the diethyl ether fraction was the most active, the petroleum ether fraction was moderately active, but the ethyl acetate fraction exhibited no activity against any of the antibacterial strains [33]. The antibacterial activity of flower essential oil is mainly attributed to its major constituent, such as phenyl ethyl alcohol (26.1%), which is a famous aromatic alcohol naturally found in fragrant flowers and reported to exhibit antibacterial activity [36]. Eucarvone (18.7%) and oxygenated terpenes are also reported to be antimicrobial [7], and furfural (10.7%), an important aldehyde, is also documented to exhibit antimicrobial potential against *Escherichia coli* and *Staphylococcus aureus* [1, 30]. Furthermore, minor constituents can contribute to the antimicrobial and synergistic actions of the flower's essential oil, such as benzaldehyde (4.8%), methyl hexadecanoate (3.1%), linalool oxide (2.1%), hexahydrofarnesyl acetone (0.5%), phytol (4.7%), and tricosane (0.91%), which were earlier documented to have antimicrobial potential [1, 29].

CONCLUSIONS

In the phytochemical analysis of the essential oil of *Nyctanthes arbor-tristis* flower extracted by microwave-assisted extraction technique, 34 volatile components were identified, numerous of which were aromatic and biologically active, with alcohols and monoterpenes found as the dominant classes. This potent volatile oil showed promising activity against different ordinary and multidrug-resistant bacterial strains and hence should further be tested for clinical studies and other bioactive properties as well.

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CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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