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Chromatographic and spectroscopic determination of solvent-extracted *Lantana camara* leaf oil

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Abstract

Objective: This study aimed to determine the chemical profile of *Lantana camara* leaf oil.
Methods: The essential oil was extracted from dried leaf samples using the Soxhlet extraction method. The oil was separated from the solvent and the bioactive compounds were identified using gas chromatography–mass spectroscopy (GC-MS) and Fourier transform infrared spectroscopy (FTIR). The identified peaks in the mass spectrum were matched with the database of the US National Institute of Standards and Technology (NIST) library.
Results: The FT-IR results indicated the presence of alcohols, carboxylic acids, phenols, alkanes, ketones, and primary amine compounds. GC-MS identified 43 compounds representing 95% of the total leaf essential oil components. Some of the major isolated compounds included a pyrrolizine; 1-dodecanol; 1,2-nonadecanediol; phytol; 1,3-dioxolane; 4-undecene, 9-methyl, (Z)-; 1-eicosanol; and imidazole.
Conclusions: The identified constituents of the extracted oil have established pharmacologic and insecticidal activities, and these compounds are also used in the drink, food, and cosmetic industries. This extract is highly valuable for the medical treatment of various ailments.

Keywords

Lantana camara, phytochemical profile, biological property, gas chromatography–mass spectroscopy, Fourier transform infrared spectroscopy, oil extract

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Introduction

Plants are a source of many bioactive compounds that play a significant role in the treatment of various ailments.[1] They are rich in secondary metabolites with

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outstanding biological activities that are suited to different applications and treatments. These secondary metabolites have a variety of structures and physicochemical properties.

Lantana camara (Verbenaceae) is a strong, evergreen, aromatic flowering shrub with a distinct fragrance[2] The plant grows in tropical and subtropical regions of the world.[3] In Ethiopia, *L. camara* is known locally as "Yewef kollo" and is used widely in traditional medicine.[4] It was first introduced into Ethiopia for ornamental purposes because of its attractive fragrance and flower colors. *L. camara* can grow up to 4 m high under normal conditions, but it can grow as high as 13 m when supported. It grows well under a variety of agroclimatic conditions.[5]

L. camara is a medicinal plant that contains a large number of phytochemical compounds.[6] The fresh leaves are crushed and boiled with water for the treatment of eczema, tinea, dermatitis, traumatic injuries, diarrhea and other bowel disorders, ulcers, cholera, and wound bleeding.[7,8] Leaf powder and oil extract have been shown to exhibit antimicrobial, anti-inflammatory, antioxidant, antihypertensive, and antitussive effects.[5] The plant roots and several other parts of the plant have been used to treat malaria, rheumatism, and certain skin ailments, among others.[9] The therapeutic value of *L. camara* is attributable in the most part to bioactive compounds such as flavonoids, alkaloids, tannins, saponins, steroids, glycosides, phenyl-containing compounds, and triterpenoids.[10]

L. camara extracts are extremely toxic toward pests and exert a highly repellent effect on weevils in stored grains.[11] In rural areas in Ethiopia, farmers have been using these extracts as insecticides during grain storage to prevent infestations by weevils and other insect pests. *L. camara* leaf extracts also possess larvicidal activity, while oil extracts from the flowers have repellent activity against mosquitoes.[12]

In recent years, chromatographic and spectroscopic analyses have played a key role in pharmaceutical and biomedical investigations.[13] Researchers have used these methods for numerous studies of plants to analyze their physicochemical composition, particularly techniques such as gas chromatography–mass spectroscopy (GC-MS) and Fourier transform infrared spectroscopy (FTIR), which are highly sensitive. Even though the chemical composition of the essential oils extracted from *L. camara* growing in different countries has been reported, that of *L. camara* grown in Ethiopia has not been studied to date.

The objective of the present study was to analyze the volatile phytochemical constituents of oil extracted from *L. camara* leaves collected at Bezawit Palace, Ethiopia using chromatographic and spectroscopic techniques.

Materials and methods

Collection of plant materials

Healthy, mature *L. camara* leaves were collected from the forest at Bezawit Palace, Bahir Dar, Ethiopia in April 2019. The leaves were cleaned and then dried in the shade for 13 days. The leaves were subsequently ground to a powder using a grinder.

Extraction of Lantana camara oil

The Soxhlet extraction method[14] was used to extract the oil from *L. camara* leaves. Approximately 50 g of the powdered *L. camara* leaf was placed into a cellulose thimble and put inside the Soxhlet apparatus. The oil extracting solvent comprised 500 mL of either methanol, ethanol, or ethyl acetate. Oil extraction was performed at the solvent boiling temperature and the

extraction time was 5 to 6 hours. The solvent extract was concentrated with a rotary evaporator at reduced pressure to achieve maximum yield of the essential oil. The extracted oil was stored at 4°C in a refrigerator and dried under anhydrous Na_2SO_4 for further analysis.

Phytochemical investigation of oil extract

Phytochemical methods are used as a tool to study the different secondary metabolites present in plants that may be responsible for their medicinal properties.[15] The oil extract and powdered leaf of *L. camara* were subjected to phytochemical screening to identify the presence of active compounds such as alkaloids, tannins, saponins, flavonoids, triterpenoids, steroids, glycosides, and phenols. The presence of bioactive compounds was determined using standard methods.

Alkaloids

The method used to identify alkaloids was described by Aguoru *et al.*[16] The extracted leaf oil (0.2 g) was dissolved in 5 mL of 1% HCl and incubated in a water bath for approximately 2 minutes. The filtrate (1 mL) was treated with five drops of Dragendorff's reagent. The formation of turbidity or an orange precipitate indicated the presence of alkaloids.

Saponins

A pinch of dried leaf powder was added to a test tube containing 2 to 3 mL of distilled water and the mixture was vigorously shaken. The presence of saponins was indicated by foam formation.[17]

Tannins

The method implemented was described by Idris *et al.*[18] Extracted leaf sample (0.5 g) was dissolved in 5 mL of distilled water, then boiled gently and cooled. The solution (1 mL) was put into a test tube and three drops of ferric chloride solution were added. The sample was observed for a color change to blue-black, green, or blue-green, which indicated the presence of tannins.

Terpenes/terpenoids

The Salkowski test was used to test for the presence of terpenes.[19] The extracted leaf oil was mixed in 2 mL of chloroform, and 3 mL of concentrated H_2SO_4 were carefully added to form a layer. The sample was observed for a color change to reddish-brown at the interface, which indicated the presence of terpenes/terpenoids.

Steroids

Acetic anhydride (2 mL) was added to 0.5 g essential oil leaf extract, followed by the addition of 2 mL of H_2SO_4. The sample was observed for a color change to blue, which indicated the presence of steroid rings.[20]

Flavonoids

Diluted ammonia solution (5 mL) was added to the aqueous filtrate of the leaf extract, followed by the addition of concentrated H_2SO_4. The sample was observed for a color change to yellow, which indicated the presence of flavonoids.[20]

Glycosides

To 5 mL of the plant extract, 2 mL of glacial acetic acid and one drop each of $FeCl_3$ and concentrated H_2SO_4 were added.[21] Browning of the interface indicated the presence of glycosides.

FT-IR analysis

FT-IR analysis of the solvent-extracted oil was performed using a Shimadzu

FTIR-8400s Fourier transform infrared spectrophotometer (Kyoto, Japan). The FTIR spectrum was used to identify the functional groups of the active components based on the peaks observed in the infrared region. The extracted oil was encapsulated in a 100-mg potassium bromide (KBr) pellet to prepare a translucent sample disk and the analysis was carried out by scanning the samples across the wavenumber range of 400 to 4,000 cm^{-1}.

GC-MS analysis

GC-MS analysis was performed on a 5975C Series GC/MSD instrument (Agilent Technology, Santa Clara, CA, USA) coupled with an HP-5ms fused silica capillary column ($30\,m \times 0.25\,mm \times 0.25\,\mu m$). The GC oven temperature was first raised from 60°C to 120°C at a rate of 10°C/minute, then increased at a rate of 3°C/minute to 175°C, 5°C/minute to 205°C, 0.8°C/minute to 210°C, and then 5°C/minute to 280°C. The temperature was then held at 280°C for 5 minutes, giving a total runtime of 55.583 minutes. The temperature of the split injector was set at 240°C and the split ratio was 1:10. The essential oil (1 μL) was injected into the GC. Helium was used as the carrier gas at a constant flow rate of 1.0 mL/minute. The mass spectrometer was operated in full-scan mode with an ionization energy of 70 eV and an interface temperature of 280°C. The MS source temperature and MS quadruple temperature were 230°C and 150°C, respectively. The scan range was from m/z 30 to 550. Identification of each component was carried out by matching the retention times and mass spectra with those in the US National Institute of Standards and Technology (NIST) mass spectra database.[22] Relative abundance (% area) calculations were based on the ratio between the peak area of each compound and the sum of the peak areas of all compounds.[23]

Results and Discussion

Phytochemical analysis

Preliminary phytochemical screening tests are important for the identification of bioactive principles and may subsequently guide drug discovery and improvement. In the present study, several phytochemical constituents of *L. camara* were identified. Primary phytochemical screening of *L. camara* leaves confirmed the presence of steroids, flavonoids, tannins, glycerol, and saponins, while alkaloids were present only in the methanol and ethanol extracts. The results of the phytochemical analyses are presented in Table 1. Such phytochemicals may provide new avenues for the development of new classes of pharmaceutical, biopesticidal, insecticidal, and antimicrobial agents.[24] Previously, various organic extracts of *L. camara* leaves were reported to contain steroids, flavonoids, tannins, and fixed oil.[25] These phytochemical compounds are the top candidates conferring medicinal value to this plant. Indeed, the most abundant compounds found in all solvent extracts in the present study, including several flavonoids, glycosides, terpenoids, and alkaloids isolated from this plant, have been reported to exert diverse biological activities.[26]

FT-IR analysis

FT-IR spectroscopy revealed the presence of several functional groups such as phenols, amines, alcohols, alkenes, carboxylic acids, aliphatic compounds, carbonyl compounds, and esters. Representative FT-IR spectra of the methanol, ethanol, and ethyl acetate extracts are shown in Figure 1. Bands were observed at 3465, 3458, and 3460 cm^{-1}, which were related

Table 1. Phytochemical analysis of *Lantana camara* leaf oil.

Phytochemical	Positive indicator	Solvent extract		
		Methanol	Ethanol	Ethyl acetate
Steroids	Blue color	+++	++	+
Terpenoids	Reddish-brown color	+	−	−
Alkaloids	Orange precipitate	+	++	−
Flavonoids	Yellow precipitate	+++	++	++
Tannins	Greenish-black color	++	+	+
Glycosides	Brown interface	++	+	++
Saponins	Froth/foam formation	+++	++	+

+++, high; ++, moderate; +, weak; −, absent.

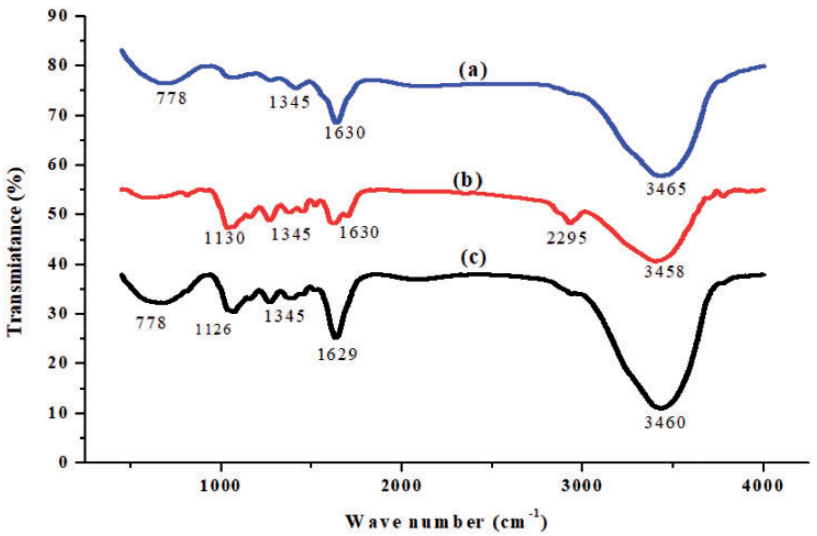

Figure 1. Fourier transform–infrared spectra of (a) methanol, (b) ethyl acetate, and (c) ethanol *Lantana camara* leaf extracts.

to the vibration of the stretching hydroxy (-OH) groups. The bands found at 1629 cm^{-1} and 1630 cm^{-1} might have resulted from the stretching vibration of the C=C groups, which include cyclic structures with a ring resonance bond that gives increased stability, and the vibration of the C=O groups of the flavonoids and lipids. The band at 1345 cm^{-1} could be related to the CH_3 and CH_2 groups of the flavonoids and aromatics. Here, the vibration would be the bending vibrations of C-H and the stretching vibration of the aromatics. The bands at 1250 cm^{-1} and 1247 cm^{-1} were related to the stretching vibration of the carboxyl group (O-H and C-O stretch), i.e., the stretching of the COOH groups in flavonoids and lipids. Bands at 1126 cm^{-1} and 1130 cm^{-1} were related to C-O stretching in the ester groups. The band at 778 cm^{-1} was due to C-C stretching vibration.[27] The band at 2295 cm^{-1} might have been related to C-H stretching vibration of the methyl and methoxy groups[28] and to

stretching vibration of the O-H groups in carboxylic acid.[29] These findings were consistent with those of earlier studies.[30,31]. The FT-IR spectra of all the oil extracts appeared similar. However, careful examination revealed several significant differences, including in the number of peaks obtained. Overall, FT-IR analysis of the methanol, ethanol, and ethyl acetate leaf extracts of *L. camara* revealed the presence of proteins, oils, fats, phenolic compounds, flavonoids, saponins, tannins, and carbohydrates as the major functional groups that are likely responsible for various medicinal properties of *L. camara*.

Chemical compounds identified in the leaf oil

The chemical composition of the leaf oil was further investigated using GC-MS, and representative chromatographs of the ethyl acetate, ethanol, and methanol extracts are presented in Figures 2, 3, and 4, respectively. The identified peaks and their contents are described in Tables 2, 3, and 4. GC-MS analysis resulted in the

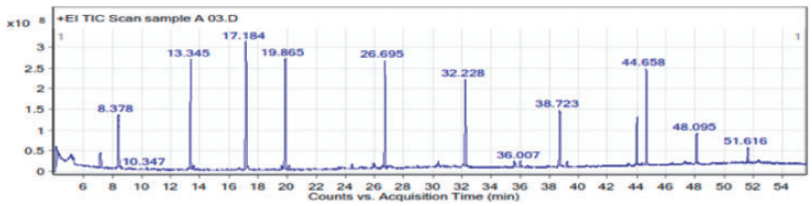

Figure 2. Gas chromatography–mass spectrometry chromatogram of an ethyl acetate extract of *Lantana camara* leaves.

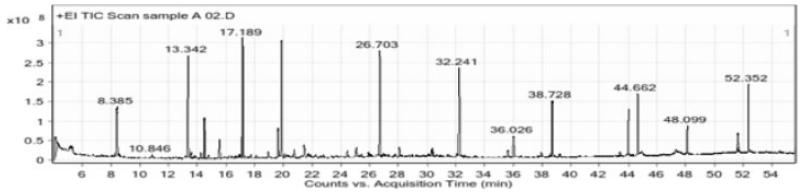

Figure 3. Gas chromatography–mass spectrometry chromatogram of an ethanol extract of *Lantana camara* leaves.

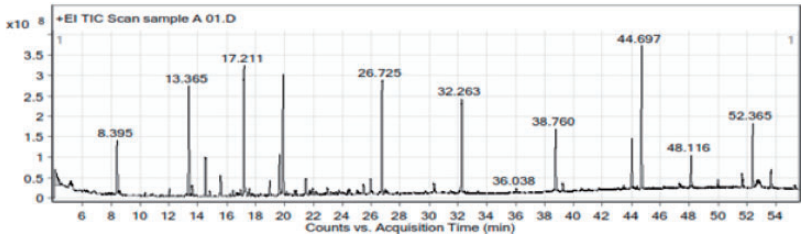

Figure 4. Gas chromatography–mass spectrometry chromatogram of a methanol extract of *Lantana camara* leaves.

Table 2. Major chemical constituents of *Lantana camara* leaf oil when using ethyl acetate for extraction.

No.	Retention time (minutes)	Compound	Peak area	Area %	Molecular weight	Molecular formula
1	8.32	4-Piperidinemethanamine	59.99	11.58	114	$C_6H_{14}N_2$
2	9.06	1-(1-Propenyl)-piperidine	1.45	0.28	125	$C_8H_{15}N$
3	13.34	1-Azabicyclo[5.1.0]octane	81.57	15.74	111	$C_7H_{13}N$
4	14.50	4-[(4-methoxyphenyl)methyl]-1, 2-oxazole-3,5-diamine	50.20	9.69	219	$C_{11}H_{13}N_3O_2$
5	14.57	4-Undecene, 3-methyl-, (Z)-	2.01	0.39	168	$C_{12}H_{24}$
6	15.50	Amphetamine	17.20	3.32	135	$C_9H_{13}N$
7	17.11	2,5-Di-tert-butylaniline	87.40	16.87	205	$C_{14}H_{23}N$
8	19.84	E-2-Tetradecen-1-ol	100.00	19.3	212	$C_{14}H_{28}O$
9	21.40	1,2,5-Oxadiazol-3-amine	6.91	1.33	85	$C_2H_3N_3O$
10	26.60	13-Methyltetradecanal	63.60	12.27	226	$C_{15}H_{30}O$
11	32.20	Hexadecanal	33.02	6.37	240	$C_{16}H_{32}O$
12	38.70	Imidazole, 2-amino-5-[(2-carboxy)vinyl]-	11.40	2.20	153	$C_6H_7N_3O_2$
13	43.80	4-(3-Pyridyl)-4-oxo-butyramide	1.21	0.23	250	$C_{12}H_{18}N_2O_2Si$
14	52.33	2-(6,7-Dimethoxy-2-oxo-3, 4-dihydro-1H-quinolin-4-yl) acetic acid	2.266	0.44	265	$C_{13}H_{15}NO_5$

No., number.

Table 3. Major chemical constituents of *Lantana camara* leaf oil when using ethanol for extraction.

No.	Retention time (minutes)	Compound	Peak area	Area %	Molecular weight	Molecular formula
1	8.37	Acetic acid, trifluoro-, 3,7-dimethyloctyl ester	48.45	6.97	254	$C_{12}H_{21}F_3O_2$
2	9.80	Chlorozotocin	54.00	7.77	313	$C_9H_{16}ClN_3O_7$
3	10.34	Pyrrolizin-1,7-dione-6-carboxylic acid, methyl(ester)	1.01	0.15	197	$C_9H_{11}NO_4$
4	11.48	Pyrimidin-2,4-dione,1,2,3,4-tetrahydro-5-methyl-1-[2-hydroxymethyl-3-dimethylamino] tetrahydrofur-5-ylPyrimidin-2,4-dione	1.09	0.16	269	$C_{12}H_{19}N_3O_4$
5	13.35	1-Undecene, 9-methyl-	69.20	9.96	168	$C_{12}H_{24}$
6	14.57	4-Undecene, 3-methyl-, (Z)-	2.01	0.29	168	$C_{12}H_{24}$
7	15.40	beta-Quinoline	25.70	3.70	129	C_9H_7N
8	17.18	2,5-Di-tert-butylaniline	100.00	14.39	205	$C_{14}H_{23}N$
9	19.87	N, N'-Tetramethylenebis	81.86	11.78	396	$C_{10}H_{24}N_2O_6S_4$
10	22.70	Paromomycin	2.17	0.31	615	$C_{23}H_{45}N_5O_{14}$
11	26.69	Z-2-Acetoxy-12-tetradecenitrile	77.00	11.08	279	$C_{17}H_{29}NO_2$
12	26.70	2-Dodecene	86.00	12.38	168	$C_{12}H_{24}$
13	32.20	Octadecane, 1-(ethenyloxy)-	65.50	9.43	296	$C_{20}H_{40}O$
14	32.21	1-Hexacosanol	75.29	10.83	382	$C_{26}H_{54}O$
15	36.00	1,5-Dinitro-3,7-diazabicyclo[3.3.1]nonane	5.61	0.81	216	$C_7H_{12}N_4O_4$

No., peak number.

Table 4. Major chemical constituents of *Lantana camara* leaf oil when using methanol for extraction.

No.	Retention time (minutes)	Compound	Peak area	Area %	Molecular weight	Molecular formula
1	8.34	4-Undecene, 5-methyl-, (E)-	43.29	5.16	168	$C_{12}H_{24}$
2	8.40	1,8-Nonadien-3-ol	47.00	5.60	140	$C_9H_{16}O$
3	13.37	Pyrrolizin-1,7-dione-6-carboxylic acid, methyl(ester)	68.00	8.10	197	$C_9H_{11}NO_4$
4	14.50	1-Pentanamine, N-(phenylmethylene)-	27.00	3.22	175	$C_{12}H_{17}N$
5	15.50	2-Oxobicyclo[2.2.1]heptane-1-carbonitrile	12.30	1.46	135	C_8H_9NO
6	17.21	2,5-Di-tert-butylaniline	99.40	11.84	205	$C_{14}H_{23}N$
7	19.60	Cyclohexanamine, N-(phenylmethylene)-	28.00	3.33	187	$C_{13}H_{17}N$
8	19.80	Cycloundecane, (1-methylethyl)-	87.10	10.37	196	$C_{14}H_{28}$
9	19.84	Imidazole, 2-amino-5-[(2-carboxy) vinyl]-	37.40	4.45	153	$C_6H_7N_3O_2$
10	20.00	1-Docosene	73.10	8.71	308	$C_{22}H_{44}$
11	21.40	4,7,10-Hexadecatrienoic acid, methyl ester	11.40	1.36	264	$C_{17}H_{28}O_2$
12	26.70	1-Dodecanol	77.60	9.24	186	$C_{12}H_{26}O$
13	30.35	Propanenitrile, 3-[1-[3-(1-pyrrolidinyl) propynyl	4.41	0.53	260	$C_{16}H_{24}N_2O$
14	32.20	1-Eicosanol	42.50	5.06	298	$C_{20}H_{42}O$
15	36.03	Phytol	3.06	0.36	296	$C_{20}H_{40}O$
16	44.70	Pyrroline, 5-butyl-2-[1,3-heptadienyl]-	100.0	11.92	219	$C_{15}H_{25}N$
17	48.10	1,2-Nonadecanediol	17.30	2.06	300	$C_{19}H_{40}O_2$
18	48.30	4-Acetyloxyimino-6,6-dimethyl-3-methylsulfanyl-4,5,6,7	2.70	0.32	341	$C_{15}H_{19}NO_4S_2$
19	51.10	6-Ethoxy-7-methoxy-1-(3-nitro-phenyl	2.39	0.28	328	$C_{18}H_{20}N_2O_4$
20	52.30	1,3-Dioxolane	55.69	6.63	74	$C_3H_6O_2$

No., peak number.

identification of 43 unique constituents in the leaf oil of *L. camara*. The oil was shown to be a complex mixture of numerous compounds, many of which are present in low quantities. The identified compounds comprised more than 95% of the total extracted oils. The percentage peak areas were taken to represent the proportion of each compound relative to the total. The major chemical components of the oil comprised a pyrrolizine, paromomycin, a pyrroline, phytol, 2-dodecene, isoquinoline, 1-eicosanol, and chlorozotocin (Figure 5). The biological properties of select components of *L. camara* essential oil based on the findings of previous studies are presented in Table 5. These include insecticidal and antimicrobial properties.

The extracts of *L. camara* leaf oil prepared using different solvents were qualitatively similar, although they did differ markedly in terms of the relative concentrations of the compounds present. The chemical composition of the essential oils extracted from Ethiopian *L. camara* agreed quite well with that previously reported in the literature,[43] with some differences in the relative quantities of the volatile compounds. Notably, there have previously been major differences reported in the chemical composition of *L. camara* leaf oils extracted from plants in certain countries.[44] The observed differences in the quality and composition of the extracted oil between studies may be attributable to factors such as genetic, climate,

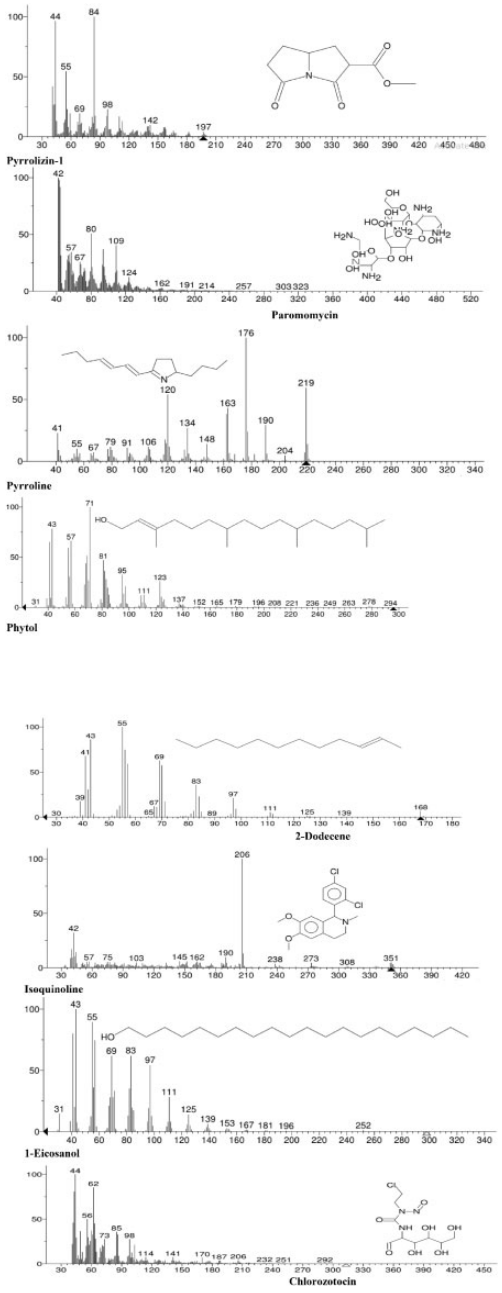

Figure 5. Mass spectra of the major chemical compounds in the oil extract of the Ethiopian *Lantana camara* leaf.

Table 5. Biological properties of individual components of *Lantana camara* essential oil.

No.	Compound	Biological properties	Reference
1	Paromomycin	Antibiotic	32
2	Pyrroline	Flavoring agent	33
3	2-Dodecene	Antibacterial	34
4	1-Eicosanol	Emollient for cosmetics	35
5	Phytol	Insecticidal, pest repellent, anti-inflammatory agent	36
6	Imidazole	Antifungal, antiprotozoal, antibacterial, antitubercular	37
7	Pyrimidine	Antiviral, anticancer, antimalarial, antifungal, antithyroid	38
8	Pyrrolizine	Antimicrobial	39
9	Amphetamine	Antibiotic, antibacterial	40
10	Isoquinoline	Insecticidal, antifungal	41
11	Chlorozotocin	Anticancer	42

No., peak number.

topographical, and seasonal variations. Among the crucial compounds identified in the present study, phytol, azabicyclo-containing compounds, and 2-dodecene have been reported in the essential oils extracted from *L. camara* leaves in other studies.[25,45]

The leaves of *L. camara* are aromatic and contain highly volatile components that confer the essential oil with many medicinal and other properties. The extracted essential oil was bright yellow. Several of the isolated compounds, namely a pyrroline, phytol, 1-dodecanol, paromomycin, 1-hexacosanol, and amphetamine are known for their pharmaceutical applications.[46] For example, paromomycin has been reported as having pain-relieving and anti-inflammatory properties, and it exhibits its antifungal activity against dermatophytes. It is also well known for its use as a preservative in drugs, foods, and cosmetics.[47] Dodecene oil, which is rich in dodecene (> 73%), has been reported to show antioxidant, antibacterial, and insecticidal activities.[48] Besides the medicinal value of

L. camara oil, the sustained demand for synthetic flavorings and fragrances for use in the pharmaceutical, food, and cosmetic industries makes this essential oil valuable for exploitation in these industries as well.

Conclusions

The Soxhlet solvent extraction method was applied to the leaves of *L. camara* harvested in Ethiopia. The leaves were found to be rich in phytol, 4-undecene, 1-eicosanol, 1-docosene, imidazole, and pyrroline. This oil extract may possess antimicrobial, insecticidal, insect repellent, and cytotoxic activities as reported for most pyrroline- and phytol-rich oils. The wide spectrum of volatile components in *L. camara* oil suggests that it may be useful for application as an antimicrobial for food preservation, as an insect repellent, and also as a flavoring agent in pharmaceutical and cosmetics products. The present study showed that the phytochemical composition of the *L. camara* extracts varied depending on the solvent used for extraction. In particular, the total flavonoid and terpenoid content varied extensively with different solvents. Methanol extracts of *L. camara* leaves contained more unique compounds than extracts obtained with the other solvents. Thus, this study affords a good foundation for further investigations of the biochemical and phytochemical functions of the various compounds identified, and the findings propose that the methanol leaf extract of *L. camara*, with its numerous bioactive compounds, is a promising source of lead compounds for future drug development. However, further studies are required to fully evaluate the potential of this oil.

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Declaration of conflicting interest

The author declares that there is no conflict of interest.

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