Tropical Journal of Pharmaceutical Research January 2018; 17 (1): 105-115
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v17i1.16

Original Research Article

Comparative evaluation of essential oils from *Lippia javanica* L leaf obtained by two methods and their effect on *Artemia salina* L

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Sent for review: 17 July 2017 *Revised accepted: 11 October 2017*

Abstract

Purpose: *To compare the chemical constituents of essential oils extracted from fresh and dried leaves of Lippia javanica by hydrodistillation (HD) and solvent-free microwave extraction methods (SFME), and evaluate their effects on Artemia salina.*
Methods: *Oil was extracted from the fresh and dried leaves of Lippia javanica by HD and SFME methods, and assayed for chemical constituents using gas chromatography-mass spectroscopy (GC-MS). The oils were tested for hatchability and preliminary toxicity on Artemia salina for 72 h. The lethal concentration required to kill fifty percent of A. salina (LC_{50}) was determined by Probit regression analysis.*
Results: *Mesityl oxide was the most abundant compound in the essential oils. Mesityl oxide content of fresh and dried leaves extracted with HD was 25.33 and 29.83 %, respectively, while SFME method yielded 19.75 and 13.46 %, respectively. The average hatching success rate of the oil was 30 % success while lethality was 100 % after 72 h. Median lethal concentration (LC_{50}) of fresh and dried leaves extracted by HD was 90.11 and 128.49 µg/mL, respectively, whereas SFME method resulted in LC_{50} of 96.52 and 101.13 µg/mL, respectively.*
Conclusion: *The results show that the essential oil yield is not significantly affected by the extraction methods used. However, the hatchability and lethality of the oils varied with the extraction method used*

Keywords: *Artemia salina, Lippia javanica, Essential oil, Hydrodistillation, Solvent-free microwave extraction*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Lippia javanica (Burm f.) Spreng. (Verbenaceae), commonly known as lemon bush is indigenous to southern and tropical Africa [1]. *Lippia javanica* leaves and other aerial parts have a strong aromatic smell, the lemon-like fragrance is often given off when these plant parts are crushed [1]. Several authors have acknowledged its uses as an insect repellant, food preservative and in the treatment of a cough, fever, wounds, diarrhoea, chest pains and asthma [1,2-4]. Several authors

[1,3,5-7] have reported the presence of some essential oil constituents such as (Z)-β-ocimene, ρ-cymene, linalool, carvone, β-cubebene, tagetenone in the leaves of *L. javanica*. The presence of such constituents in the plant seems to ascribe aromatic fragrance to it [1,8]. Various works on essential oils have demonstrated that the presence of constituents in essential oils varies from one geographical zone to another and essential oil composition is also affected by time of harvest of the plant [3,7-9].

Essential oil predominantly consists of secondary metabolites that help the plants in self-defense against microbial attack [10]. The essential oils are conventionally distilled with hydrodistillation (HD), steam distillation or organic solvent extraction methods [11]. The use of these methods contributes to the degradation and loss of some volatile compounds in addition to the longtime of distillation [10,11]. There is need to address the inadequacy of hydrolytic processes of extraction of essential oils and this raised the need for an alternative to the use of SFME. Solvent-free microwave extraction processed the oils with the combination of microwave heating and distillation and this is done at atmospheric pressure. SFME has been used to distill essential oils from *Origanum vulgare* L., *Cymbopogon citratus* (DC.) Stapf, *Mentha longifolia* (L.) L., *Moringa oleifera* Lam. and several spices species [12,13].

This study compared constituents of the essential oils generated through hydrodistillation and solvent-free microwave method of fresh and dried leaves and their toxicity on *Artemia salina*. Brine shrimp toxicity bioassay is a preliminary method of screening plant constituents for cytotoxicity and is an indicator for potential antitumor, anticancer and antimicrobial activities [11].

EXPERIMENTAL

Plant material

Fresh *L. javanica* leaves were harvested along the main access road to Hogsback. Hogsback is located at 32.5952° S, 26.9323° E, close to Alice in the Eastern Cape Province, South Africa. Prof Maroyi of the Department of Botany, University of Fort Hare authenticated the plant and voucher specimen deposited in the Griffen Herbarium (UFH), University of Fort Hare. The leaves were separated from their branches and rinsed with distilled water, and then separated prior to extraction into fresh and dried samples. One of the dried samples was dried in an oven before analysis.

Determination of dry leaf weight

The dry weight of dried leaves was determined with 300 g of fresh leaves of *L. javanica* placed in an oven at 25 ^{o}C for 48 h. The weight was obtained through evaporation of moisture from leaves of the plant. This was generated by subtracting the weight of the leaves after drying from the weight prior to oven drying of the leaves and then measured in percent.

Solvent-free microwave extraction (SFME) of essential oil

Solvent-free microwave extraction was carried out according to the method employed by Kayode and Afolayan [10]. Two hundred grams each of *L. javanica* fresh and dried leaves at different times were set into the reactor without the addition of water or any solvent. The exhaustive extraction of the essential oil was obtained at 40 min.

Hydrodistillation

Two hundred grams each of fresh and dried *L. javanica* separately were hydrodistilled for 3 h in an all-glass Clevenger apparatus, with heat supplied to the heating mantle (30 ^{o}C) and the essential oil was extracted with 4 litres of water for 3 h (until no more essential oil was recovered). The essential oil was collected and analysed immediately and this was done in accordance with the description of Okoh and Afolayan [11].

Determination of yield of essential oil

The yields of essential oils were determined using the method adopted by Adeogun et al [15] with slight modification. The quantity of the oil was obtained by deducting the weight of the dried essential oil over anhydrous sodium sulfate from the weight of the leaves prior to extraction and expressed as a percentage.

Gas chromatography-mass spectroscopy (GC-MS)

The GC-MS procedure used by Kayode and Afolayan [10] was adopted for this study. Agilent 6890 GC was coupled to an Agilent 5975 MSD with a Zebron-5MS column (ZB- 5MS 30 m x 0.25 mm x 0.25 lm) (5 % phenylmethyl-polysiloxane). GC grade helium was used as a carrier gas at a flow rate of 2 mL/min; splitless 1 µL injections were used. Injector temp 280 ^{o}C; source temp 280 ^{o}C. Oven temp was 70 ^{o}C, ramp 15 ^{o}C/min to 120 ^{o}C, ramp at 10^{o}C/min to 180 ^{o}C then ramp at 20 ^{o}C/min to 270 ^{o}C and

hold for 3 min. Data was gathered with Chem-station.

Hatchability test on *Artemia salina*

This test was conducted to ascertain if essential oils from *Lippia javanica* leaves has the potential of hatching the eggs of *Artemia salina*. Ten eggs were put into a 30-mL capacity sterile petri dish, each containing a freshly prepared mixture of the essential oil solubilized with Dimethyl sulfoxide, and seawater (pH: 7.91) at varying concentration of 31.25, 62.5, 125, 250, 500, 1000 µg/ml. Different control samples consisting of 0.1 % dimethyl sulphoxide (DMSO, i.e., 0.1 ml DMSO in 100 ml sea water), sea water and chloramphenicol were prepared on an individual base. The nauplii were counted in every 12 hours for 72 hours. The procedure for the experiment was carried out in a sterile petri-dish in triplicate, with access to illumination, and this followed the method adopted by Kayode and Afolayan [10] and Okoh and Afolayan [11].

Lethality test on *Artemia salina*

The test was performed to determine the effect of essential oil from fresh and dried leaves of *L. javanica* on brine shrimp nauplii. This was performed based on the method employed by Kayode and Afolayan [10] and Okoh and Afolayan [11] using brine shrimp eggs obtained from Ocean Star International, USA. The shrimp eggs were hatched in seawater for 48 h at 28 ^{o}C with constant illumination prior to the addition of the test oil. The nauplii were attracted to one side of the vials with illumination. The stock solution of the essential oil from *L. javanica* was prepared by dissolving 100 mg of the essential oil in 1.0 mL of DMSO. From the stock solution, 100 ml of different concentrations of 31.25, 62.5, 125, 250, 500, 1000 µg/mL of the essential oils was prepared with natural seawater. Control samples without essential oil consisting of 0.1 % DMSO in sea water, sea water without essential oils and chloramphenicol were prepared differently. Ten nauplii were added to each test oil and each control sample and the dead nauplii were counted in every 12 hours for 72 hours. The test was conducted in triplicate with a sterile petri-dish, with access to illumination.

Statistical analysis

Statistical analysis was done using one-way analysis of variance (ANOVA) followed by Duncan multiple range test and probit regression analysis of the LD_{50} using SPSS. Significance of difference was set at $p < 0.05$.

RESULTS

Dry leaf weight

The dry weight of the leaf after oven-drying was 49.37 %.

Essential oil yield

The yield of the essential oils from the leaf of *L. javanica* extracted with hydrodistillation and solvent-free microwave extractor is depicted in Table 1.

Table 1: Yield of essential oils of fresh and dried leaves of *L. javanica*

Mode of extraction	Fresh leaf (%)	Dried leaf (%)
Hydrodistillation	1.52	0.42
Solvent free microwave extractor	1.23	0.34

Chemical constituents of the essential oil

The GC-MS analyses as shown in Table 2, revealed the presence of 44 and 56 compounds in oils extracted from fresh and dried leaves of *L. javanica* through hydrodistillation method respectively, while 48 and 44 compounds were extracted from fresh and dried leaves of *L. javanica* through solvent-free microwave extraction method. The Table also showed that 19 compounds occurred across the four test oils assayed.

Effect of the essential oils of different concentration on hatchability of *Artemia salina* at different time of exposure

Table 3 show the percentage hatchability of *A. salina* exposed to different time range. The percentage hatchability range from 10 % after 12 hours exposure at a concentration of 500 µg/mL to 73.33 % after 72 h at a concentration of 31 µg/mL. The figures show the significant difference ($p<0.05$) based on DMRT on the hatchability activities of the different essential oils from fresh and dried leaves of *L. javanica* through hydrodistillation and solvent free microwave extraction methods with chloramphenicol treated samples, natural sea water and 0.1 % DMSO.

Effect of lethality of the essential oils at different concentration on nauplii of *Artemia salina* at different time of exposure

The mortality of the nauplii of *A. salina* after exposure to different concentrations of the test oils as depicted in Table 4, which shows that the

Table 2: Chemical constituents of the essential oil of fresh and dried leaves of *Lippia javanica* using hydrodistillation and solvent free microwave methods

Essential oil constituent	Fresh leaves SFME (%)	Dried leaves SFME (%)	Fresh leaves HD (%)	Dried leaves HD (%)	KI
Monoterpene hydrocarbons					
α-Pinene	0.08	0.12	0.30		940
Sabinene	2.50	0.19	4.25	0.33	955
β-Myrcene	5.03	5.17	7.48	5.89	961
α-Phellandrene	3.12	2.59	5.03	3.71	968
ρ-Cymene	1.49	1.31	2.22	1.09	976
α-Ocimene		0.05	0.13	0.06	983
3-tert-butylphenol,m-tert-butyl	6.99	8.05	8.77		1056
β- Bourbonene	0.28	0.21	0.55	0.24	1094
Ɣ-Terpinene	0.16	0.15	0.13	0.11	993
Caryophyllene	2.22	1.18	2.37	3.12	1105
4-tert-Butyphenol	0.15			5.36	1080
Artemisia triene	0.09				1199
Geranyl-p-cymene	0.09				1175
(R)-α-Pinene			0.49		940
Camphene			0.11	0.05	947
β-Thujene		1.63	1.09	1.82	978
β- Phellandrene			3.19		979
Cis- Verbenone			0.15		1052
3,9- Epoxy-p-mentha-1,8 (10)-diene naphthalene, 1,2,3,4, 4a, 5, 6, 7-octahydro-4a-methyl			15.94	10.90	1062
Benzene, 1-ethoxy-4-ethyl-			0.06		1081
2-Butanone, 4-phenyl				0.19	1037
Eugenol				0.19	1101
Isopiperitenone			0.10	0.08	1098
Oxygenated monoterpenes					
Mushroom alcohol		0.15		0.10	-
Linalool	4.76	3.92	4.71	2.00	1004
(+) Borneol		0.72		0.09	1036
Terpene-4-ol	0.37	0.41			1039
L-. α-Terpineol	1.32	0.85	1.13	0.50	1043
Isoborneol	0.70		0.54		1038
(-) - Terpinene-4-ol	0.69			0.60	1074
Geraniol	0.08				1499
Phytol	0.21				1232
3,4-dimethylbenzyl alcohol			0.19	0.11	-
Humulene			0.17	0.19	1114
6-epi-shyobunol			0.07		1132
3 - Allylguaiacol	0.63	0.34	0.18	0.05	1101
Isoborneol	0.70		0.54		1038
Ketones					
Artemia ketone		0.27			988
Thujone	3.02	6.98		0.23	1010
Mesityl oxide	25.33	29.85	19.75	13.46	-
Isophorone			1.19		1103
Aldehydes					
2-Hexenal, (E)	0.07		0.20	0.15	-
Sequiterpene hydrocarbons					
Naphthalene, 1,2,3,4, 4a, 5, 6, 7- octahydro-4a-methyl	12.85	11.36			1060
Copaene	0.49	0.44	0.61	0.53	1090
1,4,7, - Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z-	0.16	0.09			1114
β- Panasinsene		0.08			1116

Table 2: Chemical constituents of the essential oil of fresh and dried leaves of *Lippia javanica* using hydrodistillation and solvent free microwave methods ***[continued…]***

Essential oil constituent	Fresh leaves SFME (%)	Dried leaves SFME (%)	Fresh leaves HD (%)	Dried leaves HD (%)	KI
ϒ- Muurolene		0.12	0.16		1118
β- Cubebene		1.47			1121
Bicyclogermacrene	0.34	0.30	0.28	0.34	1125
δ-Cadinene	0.24	0.33	0.34	1130	0.24
Germacrene D-4-ol		0.29		0.39	1147
Caryophyllene oxide	0.59	0.31	0.61	0.43	1150
α- Cadinol		0.33	0.20	0.20	1163
β- Maaliene	0.19	0.10			1170
Alloaromadendrene	0.15		0.12	0.15	1116
Gemacrene D	2.44		2.43	2.90	1121
Nerolidol 2	0.07		0.07		1137
Neryl (S)-2-Methylbutanoate	0.05				1139
Cycloheptane, 4-methylene-1-methyl-2-(2-methyl-1-propen-1-yl)-1-vinyl	0.38				1174
Chamigran-9-one, 2, 10-dibromo-3-chloro	0.04		0.08		1244
Binapacryl	0.19		0.16		1295
2(1H)-Naphthalenone, 4a, 5, 6, 7, 8, 8a-hexahydro-4a, 8a-dimethyl-, cis-			0.24		1162
Sequiterpene hydrocarbons					
Chamigran-7-en-9-ol, 2, 10-dibromo-3-chloro			0.08		1229
Alloaromadendrene oxide –(1)				0.08	1155
Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethyl)-,[1R-(α.,7.β.,8a. α)]				0.22	1163
Esters					
Isobornyl acetate		0.21			1087
L-Proline, N-trifluoroacetyl-,3,3,3-pentafluoropropyl ester		0.20			1107
6-((E)-2-Methylbut-2-enamido) hexyl (E) methylbut-2-enoate		0.05			1261
Sorbic acid vinyl ester	0.85		1.43	0.60	-
Linolenic acid	0.14				1145
3-Methylbut-2-enoic acid, 2,3,4,6-tetrachlorophenyl ester	0.08				1207
3-Methylbut-2-enoic acid, 2,3,4,6-tetrachlorophenyl ester	0.10				1299
(E) -2-Isopropyl-5-methylphenyl 2-methylbut-2-enoate			0.15		1289
Nortilidine		0.15			
1,4-Cyclooctadiene	2.22				-
Cyclopentane, (3-methylbutylidene)	4.79				1064
2,4-Hexadiene, 2,5-dimethyl	0.33				-

Table 2: Chemical constituents of the essential oil of fresh and dried leaves of *Lippia javanica* using hydrodistillation and solvent free microwave methods ***[continued…]***

Essential oil constituent	Fresh leaves SFME (%)	Dried leaves SFME (%)	Fresh leaves HD (%)	Dried leaves HD (%)	KI
1,4-Cyclohexadiene, 1-ethyl			1.77	-	
(7R, 8S)-cis-anti-cis-7,8-eposytricyclo [7.3.0.0 (2,6] dodecane			0.27	1193	
1H-Pyrazole, 1,3,5-trimethyl			0.06	0.07	
3-Tetradecen-5-yne, (E)			0.06	1228	
4-Cyclopropylcyclohexane				0.08 1019	
1,2-Benzenediol, o- (3-methylbut-2-enonyl)-		0.60		1201	
9-Borabicyclo [3.3.1] nonane, 9-(3-methoxycyclohexyl) oxy-		0.30		1317	
Cobalt, (eta-3-trimethylgermylcyclooctenyl)-1,5-cyclooctadiene	0.08			1199	
Methyl-2,3-anhydrous-5-p-nitrobenzoyl-α-d-lyxofuranoside			0.07	1228	
Cyclohexyldichlorophosphine			0.15	-	
Furazan,3-(dimethylaminomethylenamino-4-(1,2,4-triazol-3-yl)			0.10	-	

mortality ranged from 13.33 % at 12 h exposure at a concentration of 31µg/mL to 100 % after 72 h exposure at a concentration of 1000 µg/mL. There is a significant difference ($p < 0.05$) based on DMRT on the mortality activities of the test oils as against the mortality of *A. salina* in samples treated with chloramphenicol, natural sea water and 0.1 % DMSO differently.

Lethal concentration (LC_{50}) of essential oils

Table 5 shows the concentration required to kill half of the population of test *A. salina*. The LC_{50} of fresh and dried leaves extracted through hydrodistillation method is 90.11 µg/mL and 129.14 µg/mL respectively while the LC_{50} of fresh and dried leaves extracted through solvent-free microwave extraction methods are 96.52 µg/mL and 101.13 µg/mL respectively. The study also takes cognizance of the LC_{50} of an antibiotic drug, chloramphenicol, which has an LC_{50} of 283.26 µg/Ml. Fresh leaves SFME (%) and dried leaves SFME (%): composition of essential oils of fresh and dried leaves of *L. javanica* obtained using solvent free extraction method respectively. Fresh leaves HD (%) and dried leaves HD (%): The composition of essential oils of fresh and dried leaves of *L. javanica* obtained by hydrodistillation method, respectively. Compounds < 0.05 % are not listed.

DISCUSSION

Several authors have documented the chemical constituents of essential oils from leaves of *L. javanica* [1,3,6) but none has carried out a comparative evaluation of the composition of the essential oils from fresh and dried leaves of *L. javanica* using hydrodistillation and solvent free microwave extraction methods and their activities on *A. salina*. The dry weight of the leaf was 49.37%; Alakali *et al* [16] observed that drying of plant samples above 50^{o}C can affect the quality of the sample. The test plant leaves were dried at 30^{o}C and this falls under what was established by Alkali *et al* [16].

This study was able to establish that the yield of the fresh leaves extracted with both SFME and HD had a higher yield than the yield of dried leaves through both SFME and HD. The higher yield in fresh leaves of the *L. javanica* negates what was reported by Silva *et al* [17], with the dried leaves of *Eucalyptus cinera* having a higher yield than the fresh ones. The yield of the essential oils of the leaf part of this plant was higher with hydrodistillation method compared with solvent-free microwave extraction method.

This observation corroborates the work of Kayode and Afolayan [10], they posited that the seed of *Moringa oleifera* had higher essential oils with HD than SFME. The work of Lucchesi *et al* [12] substantiates the high yield in oils extracted

Table 3: Hatchability effect of essential oils from *Lippia javanica* on *Artemia salina* Nauplii

	Hatchability success rate (%)					
Test sample	***31µg/mL***	***63µg/mL***	***125µg/mL***	***250µg/mL***	***500µg/mL***	***1000µg/mL***
			12 h			
HDLD	46.67±3.33^{c}	33.33±3.33^{b}	26.67±3.33^{a}	23.33±3.33^{b}	23.33±3.33^{c}	00.00±0.00^{a}
MCLD	40.00±0.00^{bc}	33.33±3.33^{b}	26.67±3.33^{a}	20.00±0.00^{ab}	20.00±0.00^{bc}	00.00±0.00^{a}
MCLF	30.00±5.77^{c}	26.67±3.33^{ab}	20.00±0.00^{a}	13.33±3.33^{a}	13.33±3.33^{ab}	00.00±0.00^{a}
HDLF	36.67±3.33^{bc}	30.00±0.00^{b}	23.33±3.33^{a}	16.67±3.33^{ab}	10.00±0.00^{a}	00.00±0.00^{a}
DMSO	20.00±3.33^{bc}	20.00±0.00^{a}	20.00±0.00^{a}	20.00±0.00^{ab}	20.00±0.00^{bc}	20.00±0.00^{b}
SW	43.67±3.33^{c}	43.67±3.33^{c}	43.67±3.33^{b}	43.67±3.33^{c}	43.67±3.33^{d}	43.67±3.33^{c}
CP	76.67±3.33^{d}	76.67±3.33^{d}	76.67±3.33^{c}	76.67±3.33^{d}	76.67±3.33^{e}	76.67±3.33^{d}
			24 h			
HDLD	60.00±0.00^{c}	46.67±3.33^{abc}	40.00±0.00^{b}	33.33±3.33^{b}	16.67±3.33^{a}	00.00±0.00^{a}
MCLD	53.33±3.33^{bc}	50.00±0.00^{bc}	43.33±3.33^{c}	36.67±3.33^{bc}	20.00±0.00^{a}	10.00±0.00^{a}
MCLF	40.00±0.00^{a}	36.67±3.33^{ab}	23.33±3.33^{a}	20.00±0.00^{a}	16.66±3.33^{a}	6.67±3.33^{a}
HDLF	40.00±0.00^{a}	33.33±3.33^{b}	30.00±0.00^{ab}	20.00±0.00^{a}	13.33±3.33^{a}	10.00±0.00^{a}
DMSO	46.67±3.33^{ab}	46.67±3.33^{abc}	46.67±3.33^{c}	46.67±3.33^{cd}	46.67±3.33^{b}	46.67±3.33^{b}
SW	53.33±8.81^{bc}	53.33±8.81^{c}	53.33±8.81^{c}	53.33±8.81^{d}	53.33±8.81^{b}	53.33±8.81^{b}
CP	76.67±3.33^{d}	76.67±3.33^{d}	76.67±3.33^{d}	76.67±3.33^{e}	76.67±3.33^{c}	76.67±3.33^{c}
			36 h			
HDLD	63.33±3.33^{c}	50.00±0.00^{b}	43.33±3.33^{b}	40.00±0.00^{b}	23.33±3.33^{a}	00.00±0.00^{a}
MCLD	60.00±0.00^{c}	56.67±3.33^{b}	50.00±0.00^{bc}	36.67±3.33^{b}	23.33±3.33^{a}	13.33±3.33^{b}
MCLF	46.67±3.33^{a}	40.00±0.00^{a}	30.00±0.00^{a}	26.67±3.33^{a}	20.00±0.00^{a}	10.00±0.00^{b}
HDLF	46.67±3.33^{a}	36.67±3.33^{a}	33.33±3.33^{a}	23.33±3.33^{a}	23.33±3.33^{a}	16.67±3.33^{b}
DMSO	50.00±0.00^{b}	50.00±0.00^{b}	50.00±0.00^{bc}	50.00±0.00^{c}	50.00±0.00^{b}	50.00±0.00^{c}
SW	56.67±3.33^{bc}	56.67±3.33^{b}	56.67±3.33^{c}	56.67±3.33^{c}	56.67±3.33^{b}	56.67±3.33^{c}
CP	73.33±3.33^{d}	73.33±3.33^{c}	73.33±3.33^{d}	73.33±3.33^{d}	73.33±3.33^{c}	73.33±3.33^{d}
			48 h			
HDLD	70.00±0.00^{b}	56.67±3.33^{ab}	50.00±0.00^{a}	46.67±6.67^{ab}	26.67±6.67^{ab}	6.67±3.33^{a}
MCLD	66.67±3.33^{b}	60.00±0.00^{abc}	46.67±3.33^{a}	36.67±3.33^{a}	20.00±0.00^{a}	16.67±3.33^{b}
MCLF	63.33±3.33^{b}	63.33±3.33^{bcd}	50.00±0.00^{a}	36.67±3.33^{a}	33.33±3.33^{b}	20.00±0.00^{bc}
HDLF	63.33±3.33^{b}	56.67±3.33^{ab}	46.67±3.33^{a}	36.67±3.33^{a}	33.33±3.33^{b}	26.67±3.33^{c}
DMSO	53.33±3.33^{a}	53.30±3.33^{a}	53.33±3.33^{a}	53.33±3.33^{b}	53.33±3.33^{c}	53.33±3.33^{d}
SW	66.67±3.33^{b}	66.67±3.33^{c}	66.67±3.33^{b}	66.67±3.33^{c}	66.67±3.33^{d}	66.67±3.33^{e}
CP	70.00±0.00^{b}	70.00±0.00^{d}	70.00±0.00^{b}	70.00±0.00^{c}	70.00±0.00^{d}	70.00±0.00^{e}
			60 h			
HDLD	73.33±3.33^{a}	66.67±3.33^{ab}	60.00±0.00^{ab}	53.33±3.33^{b}	46.67±3.33^{bc}	40.00±0.00^{c}
MCLD	73.33±3.33^{a}	66.67±0.00^{ab}	53.33±3.33^{a}	43.33±3.33^{a}	30.00±0.00^{a}	23.00±3.33^{b}
MCLF	70.00±0.00^{a}	66.67±3.33^{ab}	53.33±3.33^{a}	40.00±0.00^{a}	36.67±3.33^{ab}	26.67±3.33^{b}
HDLF	52.33±2.49^{a}	42.00±2.80^{a}	56.67±3.33^{ab}	46.67±3.33^{ab}	30.00±5.77^{a}	13.33±3.33^{a}
DMSO	63.33±3.33^{a}	63.33±3.33^{ab}	63.33±3.33^{c}	63.33±3.33^{c}	63.33±3.33^{de}	63.33±3.33^{e}
SW	70.00±0.00^{a}	70.00±0.00^{b}	70.00±0.00^{c}	70.00±0.00^{c}	70.00±0.00^{e}	70.00±0.00^{e}
CP	53.33±3.33^{a}	53.33±3.33^{ab}	53.33±3.33^{a}	53.33±3.33^{b}	53.33±3.33^{cd}	53.33±3.33^{d}
			72 h			
HDLD	80.00±0.00^{b}	70.00±0.00^{b}	63.33±3.33^{b}	56.67±3.33^{bc}	33.33±3.33^{a}	20.00±0.00^{a}
MCLD	80.00±0.00^{b}	73.33±3.33^{b}	60.00±0.00^{b}	46.67±3.33^{a}	40.00±0.00^{ab}	33.33±3.33^{b}
MCLF	80.00±3.33^{b}	73.33±3.33^{b}	66.67±3.33^{bc}	63.33±3.33^{c}	50.00±0.00^{b}	40.00±0.00^{b}
HDLF	73.33±3.33^{b}	70.00±0.00^{b}	63.33±3.33^{b}	50.00±0.00^{ab}	36.67±6.67^{a}	33.33±3.33^{b}
DMSO	73.33±0.00^{b}	73.33±0.00^{b}	73.33±0.00^{cd}	73.33±0.00^{d}	73.33±0.00^{c}	73.33±0.00^{d}
SW	76.67±3.33^{b}	76.67±3.33^{b}	76.67±3.33^{d}	76.67±3.33^{d}	76.67±3.33^{c}	76.67±3.33^{d}
CP	50.00±0.00^{a}	50.00±0.00^{a}	50.00±0.00^{a}	50.00±0.00^{ab}	50.00±0.00^{b}	50.00±0.00^{c}

with HD compared with less yield in SFME. They also mentioned that the high yield through HD was not quite significant considering that the time of heat takes up to 4.5 h and SFME only take 30 min to achieve the same purpose, which doesn't commensurate with the yield differences.

Table 4: Lethality effect of essential oils from *Lippia javanica* on *Artemia salina* nauplii

	Lethality effect (%)					
Test sample	***31µg/mL***	***63µg/mL***	***125µg/mL***	***250µg/mL***	***500µg/mL***	***1000µg/mL***
			12 h			
HDLD	13.33±3.33^{ab}	50.00±0.00^{c}	60.00±0.00^{cd}	60.00±0.00^{c}	66.67±3.33^{b}	80.00±0.00^{c}
MCLD	23.33±6.67^{ab}	46.67±3.33^{c}	56.67±3.33^{cd}	70.00±0.00^{d}	76.67±3.33^{b}	76.67±3.33^{c}
MCLF	26.67±3.33^{b}	46.67±3.33^{c}	53.33±3.33^{c}	70.00±0.00^{d}	76.67±3.33^{b}	83.33±3.33^{c}
HDLF	20.00±5.77^{ab}	50.00±0.00^{c}	63.33±3.33^{d}	73.33±3.33^{d}	73.33±6.67^{b}	90.00±0.00^{d}
DMSO	10.00±0.00^{a}	10.00±0.00^{a}	10.00±0.00^{a}	10.00±0.00^{a}	10.00±0.00^{a}	10.00±0.00^{a}
SW	13.33±3.33^{ab}	13.33±3.33^{a}	13.33±3.33^{ab}	13.33±3.33^{a}	13.33±3.33^{a}	13.33±3.33^{a}
CP	20.00±0.00^{ab}	20.00±0.00^{b}	20.00±0.00^{b}	20.00±0.00^{b}	20.00±0.00^{a}	20.00±0.00^{b}
			24 h			
HDLD	33.33±3.33^{d}	50.00±0.00^{a}	50.00±0.00^{b}	76.67±3.33^{d}	83.33±3.33^{b}	86.67±3.33^{b}
MCLD	13.33±3.33^{a}	50.00±0.00^{b}	63.33±3.33^{bc}	66.67±3.33^{c}	83.33±3.33^{b}	86.67±3.33^{b}
MCLF	36.67±3.33^{d}	63.33±3.33^{c}	80.00±0.00^{d}	80.00±0.00^{d}	86.67±3.33^{b}	86.67±3.33^{b}
HDLF	26.67±3.33^{bcd}	56.67±3.33^{bc}	66.67±3.33^{cd}	46.67±6.67^{b}	86.67±3.33^{b}	90.00±0.00^{b}
DMSO	26.67±3.33^{bcd}	26.67±3.33^{a}	26.67±3.33^{a}	26.67±3.33^{a}	26.67±3.33^{a}	26.67±3.33^{a}
SW	20.00±0.00^{ab}	20.00±0.00^{a}	20.00±0.00^{a}	20.00±0.00^{a}	20.00±0.00^{a}	20.00±0.00^{a}
CP	23.33±3.33^{bc}	23.33±3.33^{a}	23.33±3.33^{a}	23.33±3.33^{a}	23.33±3.33^{a}	23.33±3.33^{a}
			36 h			
HDLD	33.33±3.33^{b}	63.33±3.33^{c}	76.67±3.33^{c}	83.33±3.33^{c}	90.00±0.00^{c}	96.67±3.33^{c}
MCLD	46.67±3.33^{c}	60.00±0.00^{c}	76.67±3.33^{c}	86.67±3.33^{c}	90.00±0.00^{c}	93.33±3.33^{c}
MCLF	16.67±3.33^{a}	60.00±0.00^{c}	70.00±0.00^{c}	76.67±3.33^{c}	86.67±3.33^{c}	100.00±0.00^{c}
HDLF	43.33±2.24^{c}	56.67±2.09^{c}	70.00±0.00^{c}	83.33±3.33^{c}	86.67±3.33^{c}	100.00±0.00^{c}
DMSO	33.33±3.33^{b}	33.33±3.33^{b}	33.33±3.33^{b}	33.33±3.33^{b}	33.33±3.33^{b}	33.33±3.33^{b}
SW	23.33±3.33^{ab}	23.33±3.33^{a}	23.33±3.33^{a}	23.33±3.33^{ab}	23.33±3.33^{a}	23.33±3.33^{a}
CP	30.00±0.00^{b}	30.00±0.00^{ab}	30.00±0.00^{ab}	30.00±0.00^{b}	30.00±0.00^{ab}	30.00±0.00^{a}
			48 h			
HDLD	50.00±0.00^{d}	66.67±3.33^{c}	76.67±3.33^{c}	90.00±0.00^{d}	93.33±3.33^{c}	100.00±0.00
MCLD	20.00±0.00^{a}	66.67±3.33^{c}	73.33±3.33^{c}	83.33±3.33^{c}	90.00±0.00^{c}	100.00±0.00
MCLF	36.67±3.33^{c}	73.33±3.33^{c}	80.00±0.00^{d}	86.67±3.33^{cd}	96.67±3.33^{c}	100.00±0.00
HDLF	53.33±3.33^{d}	73.33±3.33^{c}	80.00±0.00^{d}	90.00±0.00^{d}	96.67±3.33^{c}	100.00±0.00
DMSO	40.00±0.00^{c}	40.00±0.00^{b}	40.00±0.00^{b}	40.00±0.00^{b}	40.00±0.00^{b}	40.00±0.00
SW	30.00±0.00^{b}	30.00±0.00^{a}	30.00±0.00^{a}	30.00±0.00^{a}	30.00±0.00^{a}	30.00±0.00
CP	30.00±0.00^{b}	30.00±0.00^{a}	30.00±0.00^{a}	30.00±0.00^{a}	30.00±0.00^{a}	30.00±0.00
			60 h			
HDLD	43.33±3.33^{c}	80.00±0.00^{c}	86.67±3.33^{c}	93.33±3.33^{cd}	100.00±0.00^{d}	100.00±0.00^{d}
MCLD	20.00±0.00^{a}	73.33±0.33^{c}	83.33±2.89^{c}	86.67±2.24^{c}	96.67±3.33^{d}	100.00±0.00^{d}
MCLF	66.67±3.33^{d}	76.67±3.33^{c}	86.67±3.33^{c}	96.67±3.33^{d}	100.00±0.00^{d}	100.00±0.00^{d}
HDLF	70.00±0.00^{d}	80.00±0.00^{c}	90.00±0.00^{c}	100.00±0.00^{d}	100.00±0.00^{d}	100.00±0.00^{d}
DMSO	43.33±3.33^{c}	43.33±3.33^{b}	43.33±3.33^{b}	43.33±3.33^{b}	43.33±3.33^{c}	43.33±3.33^{c}
SW	30.00±0.00^{b}	30.00±0.00^{a}	30.00±0.00^{a}	30.00±0.00^{a}	30.00±0.00^{a}	30.00±0.00^{a}
CP	36.67±3.33^{bc}	36.67±3.33^{a}	36.67±3.33^{ab}	36.67±3.33^{ab}	36.67±3.33^{b}	36.67±3.33^{b}
			72 h			
HDLD	70.00±0.00^{b}	80.00±0.00^{b}	90.00±0.00^{b}	100.00±0.00^{c}	100.00±0.00^{b}	100.00±0.00^{b}
MCLD	73.33±3.33^{b}	83.33±3.33^{b}	90.00±0.00^{b}	100.00±0.00^{c}	100.00±0.00^{b}	100.00±0.00^{b}
MCLF	36.67±3.33^{a}	80.00±0.00^{b}	90.00±0.00^{b}	90.00±0.00^{b}	100.00±0.00^{b}	100.00±0.00^{b}
HDLF	46.67±3.33^{a}	86.67±3.33^{b}	90.00±0.00^{b}	93.33±3.33^{bc}	100.00±0.00^{b}	100.00±0.00^{b}
DMSO	40.00±5.77^{a}	40.00±5.77^{a}	40.00±5.77^{a}	40.00±5.77^{a}	40.00±5.77^{a}	40.00±5.77^{a}
SW	36.67±3.33^{a}	36.67±3.33^{a}	36.67±3.33^{a}	36.67±3.33^{a}	36.67±3.33^{a}	36.67±3.33^{a}
CP	40.00±0.00^{a}	40.00±0.00^{a}	40.00±0.00^{a}	40.00±0.00^{a}	40.00±0.00^{a}	40.00±0.00^{a}

This study reported the identification of 104 compounds from the fresh and dried leaves of *L. javanica* using HD and SFME. Maroyi [1] reported on his work the presence of 133 compounds in the oils of *L. javanica* regardless of the method of extraction. The number of compounds identified in the essential oils of test *L. javanica* is 44, 56, 48 and 44 in fresh and dried leaves extracted through HD and fresh and dried leaves through SFME respectively. The presence of more compounds in the dried leaves of *L.* javanica extracted through hydro distillation in

Table 5: Lethality index (indicated by LC_{50}) of test oils extracted from *L. javanica* on *Artemia salina*

Treatment	LC_{50} (µg/ml)	Regression equation	R^2 (%)	*P*-value	Chi-square
HDLD	129.14	Y= 1.25X + -2.75	80	0.0	22.19
HDLF	90.11	Y= 1.25X+ -2.25	91.2	0.1	12.58
MCLD	101.13	Y= 1X+ -2	90.6	0.8	8.33
MCLF	96.52	Y= 1X+-2	97.6	0.7	2.36
CP	283.26	Y= 0.0032X+0.9	77.2	0.15	3.76

R^2: coefficient of determination of regression equation; p-values indicate the level of significance of the regression equation; values less than 0.05 are significant and those less than 0.05 are not significant at 5 % level of probability

this study support the assertion raised by Asekun et al [18] that dried plants yielded more oils than the fresh plants. It was noted in the study that 19 compounds were present in all test oils. The essential oils were dominated by sesquiterpene hydrocarbon, considerable amounts of monoterpene and oxygenated hydrocarbons, and ketones.

The presence of mesityl oxide, ρ-terpinene, ρ-cymene, thujone and some other chemical constituents in all test oils showed that hydrolysis is not outrightly a significant factor that influences the processing of compounds in essential oils [10,19] and high compositions of some of these compounds have been attributed to ethnopharmacological, food preservation and flavouring activities. The low yield in test oils extracted from the dried leaves of *L. javanica* compared to the fresh leaves of *L. javanica* can be ascribed to the drying of the leaves before extraction. This assertion conformed to the earlier work by Rahimmalek *et al* [20] that attributed the reduced yield of essential oils from the leaves of *Thymys daenensis* was to drying of the leaves before distillation.

The presence of naphthalene, 1, 2, 3, 4, 4a, 5, 6, 7- octahydro-4a-methyl and some other compounds only in the essential oils from fresh and dried leaves of *L. javanica* through SFME method might be ascribable to the reduction in thermal and hydrolytic effect compared with hydrodistillation that that uses a great amount of water, time and energy [12].

The hatchability results obtained showed that the test oils had paltry inhibition on the *A. salina*. The hatchability successes in test oils extracted from the dried leaves of *L. javanica* through HD and SFME at 31 µg/mL were 73.33 %, followed by 70 and 52.33 % in fresh leaves through SFME and HD respectively. The significant low hatchability activity of essential oil of the fresh leaves of *L. javanica* extracted through HD might be due to the presence and yield of some inhibitory compounds such as humulene, 6-epi-shybunol, 3-Tetradecen-5-yne, (E) and Cyclopropanecarboxylic acid. Silva *et al* [17] and Bartololme *et al* [21] established the contributory effects of 3- tetradecen-5-yne and humulene as part of the compounds responsible for the anticancer and antitumoral activities of *Bidens pilosa* L. and *Casearia sylvestris* Sw.

The number of eggs hatched decreased with increasing concentration of the essential oils of *L. javanica* at a different time of exposure, mostly because of the ability of the varying concentration of test oil to diffuse across the shell of the eggs and inhibit the development of *A. salina* fetus [22]. The hatched egg increased with increase in time of exposure and the very low hatching success recorded at 12 or 24 h of exposure can be attributed to the alteration of the development of A. *salina* embryos because of the vulnerability of the organism to toxins at earlier developmental stages [23]. The rate of activities of the test oil on mortalities of *A. salina*, depend on the status and method of extraction from the leaf of *L. javanica*.

The evaluated mortality of *A. salina* at a different time of exposure showed that the fresh leaf extracted through HD had a high mortality rate, followed by fresh leaf through SFME, dried leaf through HD and dried leaf through SFME. Mortality increased with an increase in the concentration of different test oils, with mortality of fresh leaf obtained by HD at 31 µg/mL being 26.67 % while at 1000 µg/mL, it was 90.0 %, after 24 h exposure.

Kayode and Afolayan [10], and Okoh and Afolayan [11] posited that increase in concentration affected the mortality rates of essential oils from the leaves of *Mentha longiflora* and the seeds of *Moringa oleifera* on *A. salina*. They concluded that with a lower concentration, there is less mortality of *A. salina*.

LC_{50} values (fresh leaf (HD): 90.11 µg/mL, dried leaf (HD): 129.11 µg/mL, fresh leaf (SFME): 96.52 µg/mL and dried leaf (SFME): 101.13 µg/mL) obtained after 24 h exposure showed that all test oils were moderately toxic on *A. salina*. The moderate toxicity status of the test oils was deduced from Clarkson's toxicity index.

Clarkson's toxicity index placed LC_{50} < 1000 µg/mL as being toxic, LC_{50} of 500 – 1000 µg/mL as low toxic, LC_{50} of 100 - 500 µg/mL as medium toxic while LC_{50} of 100 – 500 µg/mL as being non- toxic. The $LC_{50}$was determined after 24 h based on precedence laid by Hamidi *et al* [24] and Adeogun *et al* [15] that most toxicity studies which use the Brine shrimp assay determined the toxicity by counting the survived nauplii after 24 hours of exposure to the tested sample.

The high mortality rate of the test oils corroborates the earlier work by Okoh and Afolayan [10] that recorded LC_{50} of 54.4 and 77.5 µg/ml for SFME and HD of essential oils from *Mentha longifolia* L. leaf. They concluded secondary metabolites from plants, which are active medicinally, are most times more toxic to brine shrimps. Hamidi *et al* [24] also made mention that the toxicity of plants may originate from different contaminants or from plant chemical compounds that are part of the plant.

CONCLUSION

The findings of this study show that the essential oil yield is not dependent on the method of extraction. However, solvent-free microwave method of extraction saves time and energy. The toxicity of the oil towards *Artemia salina* is moderate. The essential oils at low concentrations can be developed for enhancement of shelf life of food and for the treatment of flu and malaria.

DECLARATIONS

Acknowledgement

The authors greatly appreciate Govan Mbeki Research and Development Centre (GMRDC) of the University of Fort Hare, South Africa, for funding this project and the University of Lagos, Nigeria for granting Dr OO Adeogun study leave to utilize the funds to carry out the project at the University of Fort Hare.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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