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Original Research Article

Variation in the chemical composition of essential oils from *Artemisia afra* (Jacq) ex-Wild leaf obtained by different methods and the effect of oil extracts on *Artemia salina* L

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Abstract

Purpose: *To determine the essential oils extracted from fresh and dried leaves of Artemisia afra using hydrodistillation (HD) and solvent free microwave extraction (SFME) methods and investigate the effects of the oils on Artemia salina.*
Methods: *The essential oils were obtained from fresh and dried leaves of Artemisia afra using hydrodistillation and solvent-free microwave extraction methods. The compounds present in the oils were analysed by gas chromatography-mass spectrometry (GC-MS). The oils were assayed for hatchability and lethality activities on Artemia salina for 72 h. The lethal concentration (LC_{50}) required to kill 50 % of the population of brine shrimp by each test oil was determined using a Probit regression analysis.*
Results: *The most abundant compound was thujone (32.02 and 30.02 % in fresh leaf by HD and SFME methods, respectively) and in dried leaf (26.57 and 25.82 %, by HD and SFME methods, respectively). Mean hatchability success rate of all the oils was 70 % while lethality activity was 30 % after 72 h at the lowest concentration of the test oils. Half-maximal lethal concentration (LC_{50}) on Artemia salina was 206.97 and 406.48 µg/mL of the oil from fresh leaf obtained by HD and SFME, respectively, while for the dried leaf, it was 277.18 and 669.30 µg/mL for the oil produced by HD and SFME, respectively.*
Conclusion: *The phytoconstituents in each oil varied based on the method of extraction and the state of the leaf before and after extraction. Furthermore, the toxic activity of the oils against Artemia salina suggests that they may possess anticancer properties but this needs to be further investigated.*

Keywords: *Artemia salina, Artemisia afra, Essential oils, hydrodistillation, Solvent-free microwave extraction, Hatchability, Lethality*

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INTRODUCTION

Artemisia Afra (Jacq) ex Wild, belongs to the Asteraceae family. It is known as Umhlonyane in isiXhosa, Mhlonyane in isiZulu, Lengana in Tswana, Zengena in Southern Sotho and commonly addressed as African wormwood in English [1]. It is a medicinal plant commonly found in most part of eastern and southern Africa [2,3].

The plant has been documented to have significant importance in the treatment of colds, coughs, sore throat, pneumonia, poor appetite, diabetes, wound and malaria [4]. The plant can be applied to the preservation of food due to its antimicrobial properties [5]. The application of the plant to humans require screening for toxicity test. Some of the secondary metabolites from plants such as essential oil are regarded as being safe.

Several authors have reported that α- and β-thujone, camphor, borneal, 1,8-cineole, Ɣ-terpinene, myrtenol and carvone are some essential oil constituents present in the leaf of *A. afra* [5-8]. It has been posited that oils ascribed natural aromatic fragrance to the leaf [5]. It has been documented that the composition of essential oils from the leaves of *A. afra* varied from one geographical zone to another with consideration to the time of harvest, of the plant [9].

Essential oils are derivatives of raw materials from plant origin. The oils consist of several compounds that are complex in nature and usually in liquid, but sometimes solid [10]. They are regarded as secondary metabolites that assist plants with self-defence against microbial attack [11]. The oils are predominantly extracted through hydrodistillation, steam distillation or organic solvent extractions(s) [12]. The use of steam and solvent distillation methods have resulted in the degradation and loss of several volatile compounds. This in addition to the extended time needed for distillation [11,12]. The use of hydrolytic processes to extract oil unsettle the unsaturated components of the oil and reduce the volatile compounds present in the oil [11].

The deficiency noticed with hydrolytic processes of extraction of essential oils raised the need for an alternative. The need for an alternative brings to fore the purpose of solvent-free microwave extraction (SFME) method for extraction of essential oils. SFME is based on the combination of microwave heating and distillation and is performed at atmospheric pressure. SFME is rapid in reaching the extraction temperature for the first essential oil droplet, high output of essential oil, lower energy requirement and high purity of the oil, these make it a desirable alternative. The method has been used successfully used to extract essential oils from several plants [13-15].

This study looked at the variation in the chemical compositions of essential oils generated through hydrodistillation and solvent-free microwave method using fresh and dried leaves of *A. afra*. The effects of each test essential oil on *Artemia salina* will be looked into. The need to investigate the toxicity of the oil on *Artemia salina* is due to the use of the plants by humans. *Artemia salina* toxicity bioassay is a preliminary method of screening plant constituents for cytotoxicity and hence for potential antitumor, anticancer and antimicrobial activities [16].

EXPERIMENTAL

Plant material

Fresh *Artemisia afra* leaves with were collected at Hogsback. The plant was authenticated and deposited at Griffen Herbarium, University of Fort Hare. The leaves were rinsed with distilled water. The samples were divided into two with one used immediately for analysis while the other was dried in an oven before analysis.

Dry weight of the dried leaf

The fresh leaves of *Artemisia afra* were placed in an oven at 25 ^{o}C for 48h to determine the dry weight. The dry weight of the leaves of *A. afra* was deduced after the evaporation of moisture from the fresh leaves of the plant. This was calculated by subtracting the weight of the leaves after drying from the weight of the fresh leaves and then measured in percent.

Determination of essential oil yield

The yields of the essential oils were obtained through the deduction of the weight of essential oil, dried over anhydrous sodium sulfate from the weight of the leaves prior to extraction.

Solvent-free microwave extraction (SFME) of the essential oils of *A. afra* leaves

Solvent-free microwave extraction was carried out with a Milestone DryDIST (2004) apparatus. The multimode reactor has a twin magnetron (2 x 800W, 2450 MHz) with a maximum delivered power of 500 W in 5 W increments. A rotating microwave diffuser ensures homogeneous microwave distribution throughout the plasma coated polytetrafluoroethylene (PTFE) cavity. The temperature was monitored by an external infrared sensor. Constant conditions of temperature and water were guaranteed by the reflux of condensed water, which was achieved by a circulating cooling system at 5 ^{o}C. Two hundred grams of fresh and dried leaves respectively, were placed at different times into the reactor without addition of water or any

solvent. The exhaustive extraction of the essential oils was obtained at 40 min.

Hydrodistillation

Two hundred grams each of fresh and dried *A. afra* on different occasions were hydrodistilled for 3 h in an all-glass Clevenger apparatus in accordance with the method by of Okoh *et al* [17-18]. The essential oils was collected and analyzed immediately.

Gas chromatography-mass spectrometry (GC–MS)

The 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 µm) (5%-phenylmethylpolysiloxane). GC grade helium was used as a carrier gas at a flow rate of 2 mL/min; splitless 1 µL injections was used. Injector temp 280 ^{o}C; source temperature 280 ^{o}C. Oven temperature was 70n^{o}C, ramped at 15 ^{o}C/min to 120 ^{o}C, ramped at 10^{o}C/min to 180 ^{o}C then further ramped at 20 ^{o}C/min to 270 ^{o}C and held for 3 min. Data were gathered with Chem-station.

Hatchability test

This test was carried out to determine the effect of the essential oils from the leaves of *Artemisia afra* extracted through different methods on the hatchability of brine shrimp eggs. Twenty shrimp eggs were introduced into a 30-ml capacity sterile petri-dish, each containing a freshly prepared mixture of the essential oil and sea water at a different concentration rate of 31.25, 62.5, 125, 250, 500 and 1000 µg/ml. A negative control sample consisting of 0.1% dimethyl sulphoxide (DMSO: 0.1 mL DMSO in 100 mL sea water) without the addition of oil was prepared. Control samples consisting of 0.1% dimethyl sulphoxide (DMSO: 0.1 mL DMSO in 100 mL seawater), seawater and chloramphenicol were prepared. The experiment was carried out in triplicate based on the procedure adopted by Kayode and Afolayan [11].

Lethality test

The test was conducted to ascertain the effect of essential oils from fresh and dried leaves of *Artemisia afra* on *Artemia salina* larvae. This study adopted the methods employed by Kayode and Afolayan [11]; Okoh and Afolayan [12] for determining the lethality of essential oils on *Artemia salina*. The *Artemia salina* eggs were sourced from Ocean Star International, USA. The eggs were hatched in sea water for 48 h at 28 ^{o}C with constant illumination. The naupili (harvested *A. salina*) were attracted to one side of the vials with illumination. The stock solution of the essential oils from *A. afra* 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 and 1000 µg/mL of the essential oils was prepared with natural seawater. Different Control samples without essential oil: 0.1 % DMSO in sea water, sea water and chloramphenicol. The lethality experiments were carried out in triplicates for the essential oils studied.

Statistical analysis

Statistical analysis was done using one-way analysis of variance (ANOVA) followed by Duncan multiple range tests (DMRT), and by probit regression analysis of LC_{50} using SPSS. The data generated from ANOVA are presented as mean ± SE. Significance level was set at $p < 0.05$.

RESULTS

Dry weight

The dry weight of the leaf after it was oven-dried was 34.07 %.

Yield and physicochemical characteristics of *A. afra* leaf essential oil

The yield in percentage and physicochemical characteristics of the essential oils from the leaf of *A. afra* extracted with hydrodistillation and solvent-free microwave extraction is depicted in Table 1.

Table 1: Some properties of essential oils of fresh and dried leaves of *A. afra*

Mode of extraction	Fresh leaf (%)	Dried leaf (%)
Hydrodistillation (yield)	1.02	0.84
Solvent-free microwave extraction (yield)	0.97	0.73
Colour	Light yellow hue	
Scent	Resinous Aroma	
Strength of aroma	Strong	

Essential oil composition

The composition of the essential oils analyzed through GCMS is depicted in Table 2. The Table showed that with the use of hydrodistillation method, 36 and 37 compounds were identified from fresh and dried leaves of *A. afra* respectively. On the other hand, 47 and 50 compounds were extracted from fresh and dried

leaves of *a. afra* using solvent-free microwave extraction method.

Effect of *A. afra* leaf essential oils on hatchability of *Artemia salina*

The percentage hatchability of *Artemia salina* treated with varied concentration of essential oil in the different time range is illustrated in Figures 1-6. The percentage hatchability range from 10% after 12 h exposure at a concentration of 500µg/mL to 83.33% after 72 h at a concentration of 31µg/mL. The figure shows the significant difference ($p<0.05$) in the activities of the test oils on the hatchability success of *Artemia salina*. It also depicts the activities of chloramphenicol treated samples, natural seawater and 0.1% DMSO on the hatchability success of *Artemia salina*.

Effect of essential oils of *A. afra* on survival of *Artemia salina*

The survival assessment of *Artemia salina* treated with essential oils at different concentrations with different time of exposure is depicted in Figure 7 – Figure 12. The figures show that the mortality ranged from 13.33 % following 12 h exposure at a concentration of 31 µg/mL to 90 % after 72 h exposure at a concentration of 1000µg/mL. This test shows the variation in the mortality activities of the test oils and individual samples treated with chloramphenicol, natural sea water and 0.1% DMSO respectively on *Artemia salina*.

Table 2: Essential oil constituents (monoterpenes hydrocarbons) of fresh and dried leaves of *Artemisia afra* obtained by hydrodistillation (HD) and solvent-free microwave (MC) methods

S/no	Essential oil constituent	Fresh Leaf MC (%)	Dried Leaf MC (%)	Fresh Leaf HD (%)	Dried Leaf HD (%)	KI
1.	Santolina triene	0.48		0.06	0.38	926
2.	α- Pinene	0.10		0.05		936
3.	α-Thujene				0.10	936
4.	Camphene	1.73			1.57	947
5.	Sabinene	0.65	0.23	0.56	0.51	955
6.	β- Myrcene	0.44	2.68	0.38	0.33	960
7.	Yomogi Alcohol	0.71	0.11	0.38	0.61	962
8.	α- Phellandrene	0.12	0.41	0.08	0.19	968
9.	(+) -4-Carene	0.32		0.14	0.34	973
10.	ρ- Cymene	1.54	1.45	0.88	1.66	976
11.	Eucalyptol	12.73	3.74	9.57	12.90	980
12.	Artemisia Ketone	4.24	0.99	3.29	2.90	989
13.	Eugenol	0.13				1101
14.	Thujone	26.57	25.82	32.02	30.26	1012
15.	Isothujol	0.22		0.33		1022
16.	Camphor	13.26		11.71	13.00	1029
17.	3-p-Menthene			1.79		1031
18.	(-) – Terpinen-4-ol	1.91	0.7	1.45	1.70	1039
19.	α- Thujenal	0.28		0.27		1041
20.	L-. α- Terpineol	0.40	0.82	0.44	0.41	1043
21.	Myrtenol			0.72		1045
22.	Cis-Piperitol	0.60	0.67	1.28	1.51	1045
23.	Piperitol	0.62	0.39		0.72	1049
24.	Cis-Verbenone	0.27	2.76			1054
25.	Cis-p-mentha-1 (7). 8- dien-2-ol				0.15	1056
26.	2- Carene	0.83	0.40	0.86		1062
27.	(-)-Limonene	0.08				1098
28.	Linalool	2.07				1178
29.	Trans-Pinocamphone		2.22			1216
30.	3-tert-Butyphenol		2.47			1273
31.	3-Allylguaiacol		0.20	0.19	0.11	1362

Table 3: Essential oil constituents (oxygenated monoterpenes) of fresh and dried leaves of *Artemisia afra* obtained by hydrodistillation (HD) and solvent-free microwave (MC) methods

S/No	Essential oil constituent	Fresh Leaves MC (%)	Dried Leaves MC (%)	Fresh Leaves HD (%)	Dried Leaves HD (%)	KI
1.	Borneal	2.51	1.17	3.16	2.50	1036
2.	Chamazulene	0.06			0.06	1200
3.	Phytol	0.08				1232

Table 4: Essential oil constituents (sequiterpene hydrocarbon) of fresh and dried leaves of *Artemisia afra* obtained by hydrodistillation (HD) and solvent-free microwave (MC) methods

S/No	Essential oil constituent	Fresh Leaf MC (%)	Dried Leaf MC (%)	Fresh Leaf HD (%)	Dried Leaf HD (%)	KI
1.	Caryophyllene	0.35	0.89	0.24	0.28	1104
2.	β- copaene	0.05	0.06		0.06	1106
3.	Humulene	0.07	0.07	0.07	0.08	1113
4.	ϒ- Muurolene	0.24				1118
5.	Germacerene D	1.89		1.96	1.67	1121
6.	δ- Cadinene	0.40	0. 15	0.48	0.27	1130
7.	Nerolidol	0.14	0.05		0.16	1137
8.	Bicyclogermacrene			0.82	0.89	1140
9.	Spathulenol	0.47	0.42	0.51	0.63	1147
10.	Viridiflorol	0.19			0.17	1152
11.	Cadina – 1(2), 4 diene			0.10	0.20	1159
12.	Tau. –Muurolol	1.28				1163
13.	α-Cadinol	0.50	0.44	1.04	1.21	1166
14.	α- Gurjunene				0.65	1170
15.	α. – Copaene		0.23	0.47	0.26	1322
16.	(-) -. B. Bourbonene		0.25			1328
17.	Alloaromadendrene		0.05		0.06	1372
18.	β-Cubebene		0.87			1382
19.	β-Gurjunene		0.13			1390
20.	Cubenol		0.11			1400
21.	Caryophyllene oxide		0.60			1438
22.	Ledene oxide- (II)		0.09			1450
23.	γ-Selinene		0.10			1476
24.	Shyobunone		0.06			1547

Table 5: Essential oil constituents (esters) of fresh and dried leaves of *Artemisia afra* obtained by hydrodistillation (HD) and solvent-free microwave (MC) methods

S/No	Essential oil constituent	Fresh Leaves MC (%)	Dried Leaves MC (%)	Fresh Leaves HD (%)	Dried Leaves HD (%)	KI
1.	Pentanoic acid, 2- methylbutyl ester	0.16				1007
2.	Butanoic acid, 2 methyl-, 2-methylbutyl ester				0.20	1007
3.	3- Methyl-2-butenoic acid, oct-3-en-yl ester	`1.81				1025
4.	Acetic acid, 1, 7, 7- trimethyl-bicyclo [2.2.1] hept-2-yl ester			0.97		1087
5.	3-Methylbut-2-enoic acid, 4-nitrophenyl ester		0.14			1616
6.	5- Isopropyl-2-methylphenyl 2-methylbut-2-enoate		0.15			1639
7.	3- Methylbut-2-enoic acid, 3, 4-dichlorophenyl ester		0.53			1641
8.	3-Methyl-2-butenoic acid, 2-methyl oct-5-yn-4-yl ester		0.08			1660
9.	3- Methybut-2-enoic acid, 4-isopropylphenyl ester		0.62			1690
10.	(E)-2-Isopropyl -5-methylphenyl 2-methylbut-2-enoate		0.62			1700
11.	3- Methylbut-2-enoic acid, oct-3-en-2-yl ester		1.25			1709
12.	3-Methylbut-2-enoic acid, 2, 3, 4, 6- tetrachlorophenyl ester		0.23			1734
13.	3-Methyl-2-butenoic acid, pent-2-en-4-ynyl ester		0.13			1790
14.	3- Methyl-2-butenoic acid, cyclobutyl ester		0.14			1977
15.	Cyclohexane, (2-nitro-2-propenyl)		0.46			2109

Lethality of the essential oils on *Artemia salina*

Table 2 shows the concentration required to kill half of the population of test *Artemia salina*. The LC_{50} of fresh and dried leaves extracted through hydrodistillation method is 206.97 µg/mL and 406.48 µg/mL respectively. The LC_{50} of fresh and dried leaves extracted through SFME are 277.18 and 669.30 µg/ml respectively. The study also takes into cognizance of the LC_{50} of a control sample: Chloramphenicol, which has an LC_{50} of 283.26 µg/mL.

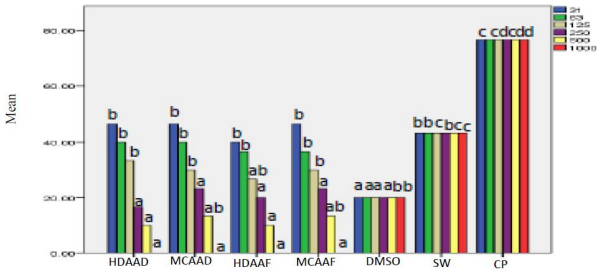

Figure 1: Hatchability after 12 h exposure

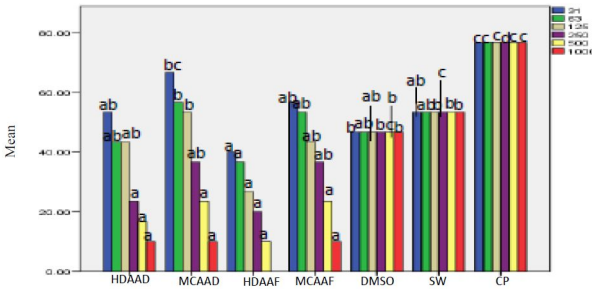

Figure 2: Hatchability after 24 h exposure

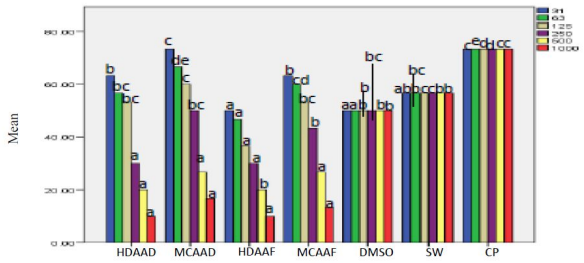

Figure 3: Hatchability after 36 h exposure

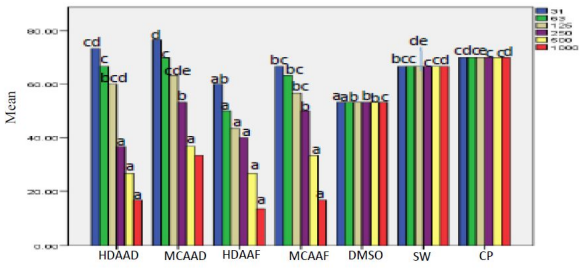

Figure 4: Hatchability after 48 h exposure

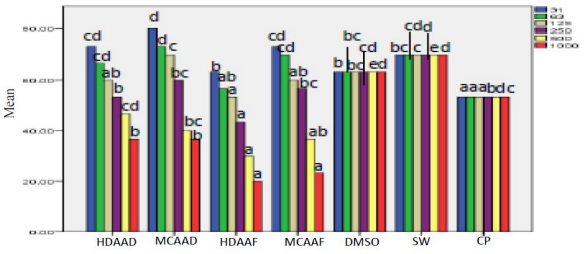

Figure 5: Hatchability after 60 h exposure

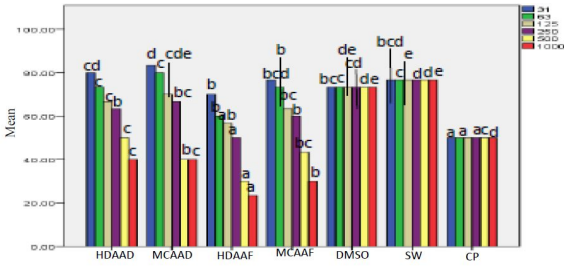

Figure 6: Hatchability after 72 h exposure

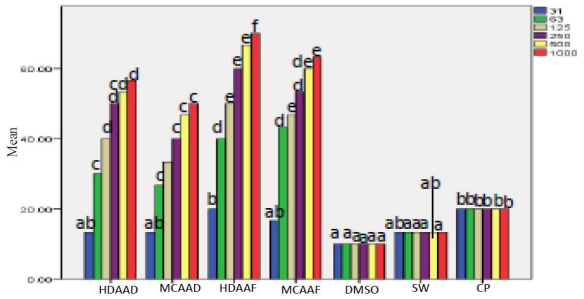

Figure 7: Lethality after 12 h exposure

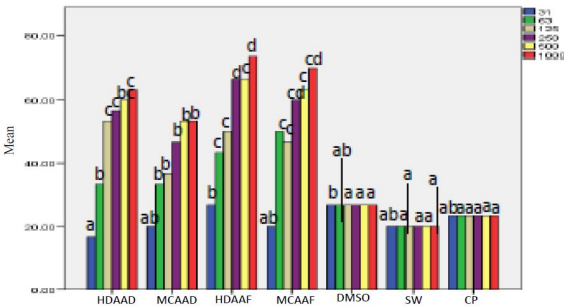

Figure 8: Lethality after 24 h exposure

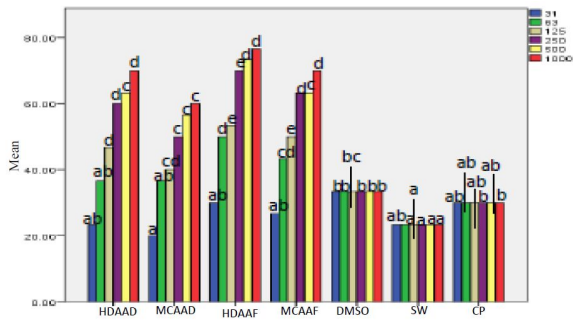

Figure 9: Lethality after 36 h exposure

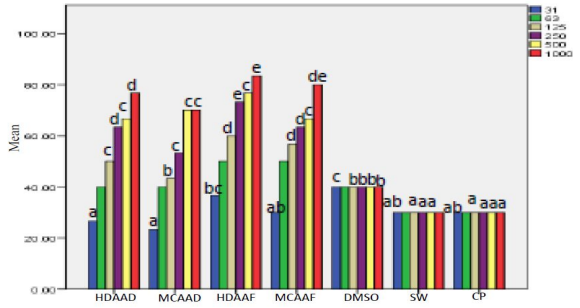

Figure 10: Lethality after 48 h exposure

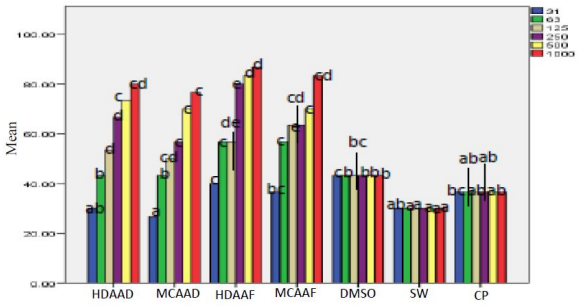

Figure 11: Lethality after 60 h exposure

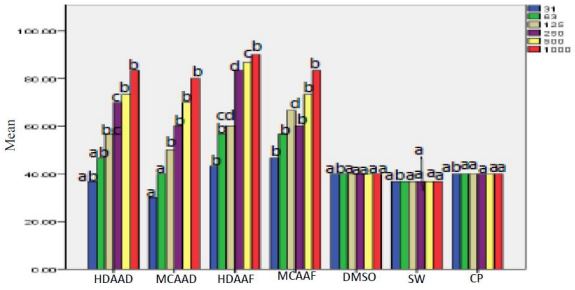

Figure 12: Lethality after 72 h exposure

DISCUSSION

The use of *Artemisia afra* (Jacq) ex Wild for medicinal purposes has been documented in African ethnopharmacology [19]. The plant alone or in combination has been acknowledged for the treatment of influenza, measles, fever, lung inflammations and food preservation [5,19].

The physicochemical properties of the studied oils were similar to previous works by Liu *et al* [19]. The loss in weight of the leaves of *A. afra* after drying in the oven indicated that there was moisture loss and this was also reflected in the yield of the essential oil from the dried leaves of the plant (dry weight of the leaf was 34.07 %). The leaf was oven-dried at 30 ^{o}C, which enable the leaf to still hold its quality compared to what was obtained in fresh leaves. This falls within the assertion raised by Alakali *et al* [20] that drying of plant samples above 50 ^{o}C can affect the quality of the sample. The assertion showed that *A. afra* dried at 30 ^{o}C would still maintain its quality. The reduced yield of essential oil from the dried leaves of *A. afra* supported what was observed by Kayode and Afolayan [11] that the dried seed of *Moringa oleifera* had lower yield, compared to the yield from the test fresh seed of *Moringa oleifera*.

This study was able to deduce that the yield of essential oils from the fresh leaves using SFME and HD was higher than the yield of dried leaves using SFME and HD. The deduction noticed in this study is at variance with the report by Silva *et al* [21], where the dried leaves of *Eucalyptus cinera* had a higher yield than the fresh ones. The yield of essential oils from the leaves of *A. afra* was higher when using hydrodistillation method than SFME methods. This corroborates the work of Kayode and Afolayan [11], where they posited that the seed of *Moringa oleifera* had higher essential oils with HD than SFME. Lucchesi *et al* [14] confirmed that there was higher yield following extraction with HD when compared with SFME. Surprisingly, they observed that the yield from HD was not quite significant when compared to SFME in terms of heating time. It took the HD (up to 4.5 h) and SFME (about 30 min) to achieve the same purpose and this couldn't explain the differences in yield.

Table 3: Lethality (LC_{50}) of test oils extracted from *A. afra* on *Artemia salina*

Treatment	LC_{50} (µg/ml)	Regression Equation	R^2 (%)	*P*- value	Chi-square
HDAAD	406.48	Y= 0.0014X + -1	47.0	0.00	35.85
HDAAF	206.97	Y= 0.0015X+ -0.9	63.0	0.00	23.12
MCAAD	669.30	Y= 0.0012X+ -1	59.2	0.06	14.38
MCAAF	277.18	Y= 0.0015X+-0.9	54.2	0.00	28.15
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. HDAAD: Dried leaves of *A. afra* extracted through Hydrodistillation Method; HDAAF: Fresh leaves of *A. afra* extracted through Hydrodistillation Method; MCAAD: Dried leaves of *A. afra* extracted through Solvent Free Microwave Extraction Method; MCAAF: Fresh leaves of *A. afra* extracted through Solvent Free Microwave Extraction Method

The phytoconstituents identified from the fresh and dried leaves of *A. afra* through HD and SFME were 73 compounds. Liu *et al* [19] documented the presence of more than 130 compounds in the oils from *A. afra*. It can be deduced from Table 2 to Table5 that there were more compounds from the oil of dried leaf samples of *A. afra* processed through hydrodistillation and SFME methods. This deduction corroborates the previous observation raised by Asekun *et al* [22] that more compounds were present in essential oils extracted from the dried leaves of *Mentha longiflora* L. compared to the fresh leaves of *M. longiflora*. There is the dominance of monoterpenoids and considerable amounts of sesquipterpenoids such as: chamazulene, bicyclogermacrene, caryophyllene oxide, caryophyllene, humulene and β-copaene, in the oils extracted across the dried and fresh leaves, supporting an earlier work by Asekun *et al* [5].

The presence of β- myrcene, α- phellandrene, ρ-cymene, eucalyptol, Artemia ketone, thujone and some other compounds in all the oils analyzed showed that the methods of extraction or state of the plant samples doesn't significantly affect the presence of some compounds in the essential oils [11,23]. The lesser yield observed in the oils from dried leaves of *A. afra* extracted with HD and SFME could be due to drying activities prior to extraction as has been suggested by Rahimmalek and Goli [24]. They asserted that drying before distillation might be responsible for the reduced yield of essential oils from the leaves of *Thymys daenensis*.

Thujone, camphor and eucalyptol were compounds were compounds identified with high percentage composition. They have been documented in the literature to possess antimicrobial, food preservation and ethnopharmacological activities [25,26]. The presence of Cis-verbenone, shyobunone and other compounds in the oils extracted through SFME method might be due to the reduction in thermal and hydrolytic effect when compared with hydrodistillation that that uses a large quantity of water, time and energy [14].

The hatchability results obtained showed that the test oils had insignificant inhibition at a lower concentration. The inhibition of *A. salina* was more pronounced with essential oils extracted from fresh leaves of *A. afra*. Hai-bin *et al* [27] established the anti-insecticidal activity of essential oils from *Artemisia lavandulaefolia* and some of the compounds noted in their work are reported in this present study.

The status of the leaf (fresh or dry) and method of extraction determined the mortality rate of essential oil on *Artemia salina*. The mortality assessment of *A. salina* can be summarized as follows: fresh leaf extracted through HD had the high mortality rate, followed by fresh leaf through SFME, dried leaf through HD and dried leaf through SFME.

The LC_{50} obtained at 24 h were 206.97, 406.48, 277.16 and 669.30 µg/ml for fresh leaf (HD), dried leaf (HD), fresh leaf (SFME) and dried leaf (SFME), respectively. The LC_{50} of the test oils showed that they are moderately toxic on *A. salina*. The toxicity status of the test oils was deduced from Clarkson's toxicity index. Clarkson's toxicity index described toxicity status as follows: non-toxic (LC_{50} > 1000 µg/mL), low toxic (LC_{50} of 500 – 1000 µg/mL), medium toxic (100 – 500 µg/mL) and toxic (0 – 100 µg/mL).

The toxicity of the essential oils obtained from *A. afra* are moderate when compared to the values obtained for other essential oils of the genus *Artemisia*. Mojarrab *et al* [28] reported LC_{50} values of 49.98 and 56.94 µg/mL for *A. incana* and *A. armeniaca*, which are less than what was obtained in this study. They opined that the presence of thujone might have contributed to the toxicity of the essential oils, as it has been reported to cause nausea, vomiting, insomnia, restlessness, vertigo and tremors.

CONCLUSION

Essential oils are generally regarded as safe for humans. The findings of this study established hydrodistillation method as a better method of extraction of essential oil than SFME method, as well as the presence of various chemical compounds in *Artemisia afra* leaves. Furthermore, the toxic activity of the oils against *Artemia salina* suggests that they may possess anticancer properties but this needs to be further investigated.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

The authors declare that the authors listed in this manuscript did this work and they would be responsible for all liabilities pertaining to claims relating to the content of this article.

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