JOURNAL OF AFRICAN RESEARCH AND DEVELOPMENT (JARD)
J.Afr.Res.Dev.(Kericho): ISSN 2517-9365
Accepted on 20th November, 2015; Reviewed on 23rd March, 2016 and Published on 20th August, 2016

Chemical composition and larvicidal activity of essential oil of *Artemisia pontica.*

By:

Joyce J. Kiplimo[a], Denis Chichir[a], Brenda Kaiyoni[a] , Jared Yugi[b] and Kebenei J. Sella[c]

[a] *Department of Physical Sciences, University of Kabianga, P.O Box 2030 Kericho, Kenya*

[b]*Department of Biological Sciences, University of Kabianga, P.O Box 2030 Kericho, Kenya*

[c]*Department of Physical and Biological Sciences, Kabarak University, Private Bag 2015, Kabarak- Kenya.*

Abstract

The essential oil of *Artemia pontica* (Asteraceae), obtained by hydro-distillation, was analysed by Gas Chromatography–Mass Spectrometry (GC–MS). Out of 72 peaks (representing 99.8% of the oil), 63 components were identified, representing 96.5% of the total oil. The oil was dominated by monoterpenes, which accounted for 89.6% of the oil. This study showed the presence of a high percentage of oxygenated monoterpenes (76.5%) of which the main constituents were *cis* thujone (33.2%), 3-thujanol (20.20%) and *trans* thujone (9.7%).

Key words**: *Artemisia pontica*, asteraceae, *cis* thujone, 3-thujanol, *trans* thujone**

1. Corresponding Author: Dr Joyce Kiplimo, Email*: jkiplimo@kabianga.ac.ke.*

2. Citation: Kiplimo et al (2016): Chemical composition and larvicidal activity of essential oil of *Artemisia pontica*. Journal of African Research and Development: (*J. Afr. Res. Dev. (Kericho)*) Vol. 1: Issue 1 (2016) pp: 1 – 7: www.jard.or.ke.

Introduction

Artemisia is a large, diverse genus of plants with between 200 and 400 species belonging to the daisy family *Asteraceae* the common names for the various species in the genus include mugwort, wormwood, and sagebrush. *Artemisia* comprises hardy herbaceous plant and shrubs, which are known for the powerful chemical constituents in their essential oils. The leaves of many species are covered with white hair. Most species have strong aromas and bitter tastes from terpenoids and sesquiterpene lactones which is an adaptation to discourage herbivory (Bora, and Sharma, 2011).

Artemisia pontica is a low growing species of *Artemisia*, Suitable as a quick spreading groundcover. It has very feathery grey-green leaves. It has insignificant flowers. The foliage has a pleasant camphor fragrance. It is a perennial plant distributed in the middle and Eastern Europe, but also common in western Siberia, western Asia and the northeastern part of America. The optimal growing conditions for the growth of *A. pontica* are, full sun or partial shade, normal or sandy or clay soil, neutral or alkaline or acid ph, Average or dry soil moisture. The appearance and characteristics include; green flower color, blooms in the mid summer, the foliage color is grey green (Tabanca *et al*., 2011). Ethnobotanically *A. pontica* is used as a deworming agent, for stomach relief, relieving gout and as a liver cleansing agent (Tabanca *et al*., 2011).

Figure 1: picture of *Artemisia pontica* growing in Kabianga location

Malaria is a serious tropical disease spread by mosquitoes. The symptoms can develop as quickly as seven day after being bitten. It is caused by a parasite known as plasmodium which is spread by female anopheles mosquitoes and injects the parasite by biting. It is a serious illness and can be fatal, it can also cause serious complications including severe anemia and cerebral malaria. It can be prevented by creating awareness risk, preventing bites, checking whether one needs to take malaria prevention tablets and by diagnosis if one develops malaria like symptoms (Packard, 2007).

Since the species of artemisia are often cited as antimalarials or febrifuges in African traditional medicine, and essential oils have been reported to possess larvicidal as well as insect repellant activity, the essential oil extracted from *A. pontica* was evaluated for its larvicidal activity.

Materials and methods

Plant collection

The leaves and twigs of *Artemisia pontica* were collected from Kabianga in Kericho County Kenya. Voucher specimen was deposited at the Department of Botany, University of Kabianga.

Isolation of volatile components

Fresh leaves of *A. pontica* were subjected to hydro-distillation in a Clevenger-type apparatus for a minimum of 4 h (fig 2). The essential oil was obtained in the yield of 2% w/w after drying over anhydrous sodium sulphate.

Figure 2: Clevenger type apparatus for hydrodistillation of essential oils

GC-MS Analysis

Samples of essential oils were diluted in methyl-*t*-butylether (MTBE) (1:100) and analysed on an Agilent GC-MSD apparatus equipped with an Rtx-5SIL MS ('Restek') (30 m x 0.25 mm i.d.,0.25 µm film thickness) fused-silica capillary column. Helium (at 0.8 mL/min) was used as a carrier gas. Samples were injected in the split mode at a ratio of 1:10 – 1: 100. The injector was kept at 250°C and the transfer line at 280°C. The column was maintained at 50°C for 2 min and then programmed to 260°C at 5°C/min and held for 10 min at 260 °C. The MS was operated in the EI mode at 70 eV, in *m/z* range 42-350. The identification of the compounds was performed by comparing their retention indices and mass spectra with those found in literature (Adams, 1995) and supplemented by Wiley and QuadLib 1607 GC-MS libraries. The relative proportions of the essential oil constituents was expressed as percentages obtained by peak area normalization, all relative response factors being taken as one.

Larvicidal screening

Larvicidal bioassay of individual plant extracts was tested against third instar larvae of Cx. *Quinque fasciatus*. The tests were conducted in glass beakers. Standard WHO (WHO, 1996) protocol with slight modifications was adopted for the study. Ten replicates and a control were run simultaneously during each trial. For control, 1.0 mL of acetone dissolved in 249 mL of dechlorinated water was used. Mosquito immature particularly early third instar larvae were obtained from laboratory colonized mosquitoes of F1 generation. Twenty healthy larvae were released in each glass beaker and mortality was observed 24 and 48 h after treatment at 1000 ppm. A total of three trials were carried out. However, when the control mortality ranged from five to twenty per cent, the observed percentage mortality was corrected by Abbott's formula (Abbott, 1925).

$$\text{Percent Mortality} = \frac{\%\ \text{test mortality} - \%\ \text{control mortality}}{100 - \%\ \text{control mortality}} \times 100$$

Results and discussion

The leaves of *A. pontica* afforded an essential oil on hydro-distillation which was analysed by gas chromatography–mass spectroscopy (GC–MS). Out of 72 peaks (representing 99.8% of the oil), 63 components were identified, representing 96.5% of the total oil. The constituents identified by GC–MS analysis, their retention times and area percentages are summarized in table 1. The oil was dominated by monoterpenes, which accounted for 89.6% of the oil. This study indicated the presence of a high percentage of oxygenated monoterpenes (76.5%) of which the main constituents were *cis* thujone (33.2%), 3-thujanol (20.20%) and *trans* thujone (9.7%).

Table 1: Chemical composition of *Artemisia pontica* leaves oil

	Compound	RI	Concentration	Method of Identification
	Monoterpenes Hydrocarbons			
1.	α-Phellandrene	9.6717	0.1204	GC-MS
2.	α – Pinene	9.7837	0.1696	GC-MS
3.	Santolina Triene	10.0748	0.4186	GC-MS
4.	Thuja-2,4(10)-diene	10.2316	0.1656	GC-MS
5.	Sabinene	10.6572	1.5556	GC-MS
6.	1,3,8-*p*- Menthatriene	11.2843	0.1964	GC-MS
7.	δ-2-Carene	11.5083	0.3065	GC-MS
8.	δ-3-Carene	15.5398	0.6414	GC-MS
9.	*o*-Cymene	11.665	0.7801	GC-MS
10.	γ- Terpinene	12.2921	0.3431	GC-MS
11.	*iso*-Sylvestrene	12.8073	0.1439	GC-MS
12.	*allo*- Ocimene	12.9193	0.1886	GC-MS
13.	Camphene	13.6136	7.8864	GC-MS
14.	Sylvestrene	15.6517	0.1958	GC-MS
	Total		**13.112**	
	Oxygenated monoterpenes			
15.	*cis*-Verbenol	12.7401	0.0742	GC-MS
16.	Octen-3-ol	10.8139	0.2645	GC-MS
17.	3-Octanol	11.1275	0.1561	GC-MS
18.	Eucalyptol	11.777	4.1713	GC-MS
19.	**cis-Thujone**	**13.188**	**33.1797**	GC-MS
20.	***trans*-Thujone**	**13.3224**	**9.7441**	GC-MS
21.	*trans*- Sabinol	13.6808	0.7668	GC-MS
22.	Camphor	13.7704	1.7097	GC-MS
23.	Sabina ketone	13.9943	0.3099	GC-MS
24.	**3-Thujanol**	**14.1287**	**20.2003**	GC-MS
25.	Terpinen-4-ol	14.3079	1.364	GC-MS
26.	Thuj-3-en-10-al	14.4199	0.2962	GC-MS
27.	α-Terpineol	14.5095	0.4841	GC-MS
28.	Myrtenal	14.5991	0.3467	GC-MS
29.	Verbenone	14.8006	0.6065	GC-MS
30.	*trans*- Carveol	14.935	0.3209	GC-MS
31.	Bicyclo[3.1.1]hept-2-en-6-ol, 2,7,7-trimethyl-, acetate, [1S-(1.alpha.,5.alpha.,6.beta.)]-	15.1366	0.2219	GC-MS
32.	Carvone	15.3158	0.5367	GC-MS
33.	Perilla aldehyde	15.7861	0.2346	GC-MS
34.	3-Thujanol acetate	15.9653	0.986	GC-MS
35.	Carvacrol	16.1445	0.5488	GC-MS
	Total		**76.523**	
	Sesquiterpene hydrocarbons			
36.	*Iso*-bazzanene	16.6148	0.2664	GC-MS
37.	α – Cubebene	16.794	0.1611	GC-MS
38.	α –Copaene	17.1747	0.2293	GC-MS
39.	*trans*-Muurola-4(14),5-diene	17.3539	0.3323	GC-MS

40.	α –Gurjunene	17.6451	0.2013	GC-MS
41.	(E)-β-Farnesene	17.7795	0.5891	GC-MS
42.	β – Copaene	17.8914	0.29	GC-MS
43.	Dauca-5,8-diene	18.093	0.1945	GC-MS
44.	α –Humulene	18.205	0.22	GC-MS
45.	*cis*-Muurola-4(14),5-diene	18.3394	0.3291	GC-MS
46.	gamma-Muurolene	18.4738	0.1402	GC-MS
47.	Germacrene D	18.5634	0.6559	GC-MS
48.	α-Duprezianene	18.653	0.1668	GC-MS
49.	Bicyclogermacrene	18.7425	0.5331	GC-MS
50.	δ-Cadinene	19.0337	0.5541	GC-MS
51.	α-Calacorene	19.3249	0.244	GC-MS
52.	*o*-Menth-8-ene	19.4593	0.0832	GC-MS
53.	β- Calacorene	19.5712	0.0743	GC-MS
54.	Amorpha-4,7(11)-diene	19.6608	0.0582	GC-MS
55.	4,5,6,7-Tetrahydroindazole-3-spirocyclohexane	19.7504	0.1835	GC-MS
56.	trans-Cadina-1,4-diene	20.3327	0.4329	GC-MS
57.	cis-Cadina-1(6),4-diene	20.4671	0.19	GC-MS
58.	beta-Gurjunene	23.7147	0.0109	GC-MS
	Total		**6.1402**	
	Oxygenated sesquiterpene			
59.	Caryophyllene oxide	19.84	0.1812	GC-MS
60.	Ledol	20.064	0.2192	GC-MS
61.	Selin-11-en-4-alpha-ol	20.6463	0.2858	GC-MS
	Total		**0.6862**	
	Diterpenes			
62.	3Z-Cembrene A	23.8715	0.0091	GC-MS
63.	Sclareoloxide	23.1548	0.0171	GC-MS
	Total		**0.0262**	
	Total		**96.49**	

Discussion

The use of natural products can be considered as an important alternative for the control of mosquitoes. Essential oils can affect insects in several ways: they may disrupt major metabolic pathways and cause rapid death, act as contact insecticides (Saxena *et al.* 1992), fumigants (Shaaya *et al.* 1997), repellents (Plarre *et al.* 1997), and deterrents or can modify oviposition. The essential oil had a good fumigation effect on adult insects. According to the results, a similar trend was observed in *A. pontica* essential oil. Similar observations about other plant extracts have also been made. For example, Wang *et al*. (2006) showed that *Artemisia vulgaris,* strongly repelled *T. castaneum. Azadirachtin* has also been demonstrated to be repellent to three stored product insects (Xie *et al.* 1995). Plant extracts may also accelerate development or interfere with the life-cycle of insects in other ways (Bell *et al.* 1990).

The insecticidal constituents of many plant extracts and essential oils are mainly monoterpenoids (Regnault- Roger and Hamraoui 1995; Ahn *et al.* 1998). Monoterpenoids are typically volatile and rather lipophilic compounds that can penetrate into insects rapidly and interfere with their physiological functions (Lee *et al.*, 2002). Due to their high volatility, they are fumigant and gaseous and might be of importance for stored-product insects (Ahn *et al.*, 1998). Therefore, insecticidal activity of *A. judaica* may be related to these components. The toxicity exhibited by essential oils and their constituent monoterpenes marks them as potentia1 alternative com- pounds to currently used fumigants. Therefore, toxicity of the *A. judaica* could be attributed to higher concentrations of camphor. Eucalyptol has also been used as an insecticide in many repellent sprays (Huang *et al.*, 2000).

Conclusion

The composition of the essential oil of *A. pontica* growing in Kenya has been analysed and its larvicidal activity investigated. The results indicate that the oil may be used in the treatment of diseases caused by the microorganisms tested. Further toxicological and clinical studies are required to prove the safety of the oil as a medicine.

Acknowledgement
The authors are thankful to ICIPE Center in Kenya for analyzing the essential oil.

References

Adams, R. P. (**2001**). Identification of essential oil by gas chromatography/quadrupole mass spectroscopy.. Allured Publishing Corporation, Carol Stream, Illinois, USA *pp* 455.

Ahn Y. I., Lee S. B., Lee H. S., Kim G. H. (**1998**). Insecticidal and acaricidal activity of caravacrol and (-thujaplicine derived from *Thujopsis dolabrata* var. *hondai* sawdust. *Journal*. *Chemical Eco1ogy*. 24 (1): 1–90.

Bell A. E., Fellows L. E., Simmonds S. J. (**1990**). Natural products from plants for the control of insect pests. p. 337–344. In “Safer Insecticides: Development and Use” (E. Hodgson, R. J. Kuhr, eds.). Marcel Dekker, New York, USA, pp. 593.

Bora, K. S. and Sharma, A. (**2011).** The genus *Artemisia*: A comprehensive review. *Pharm. Biol.*, *49*, 101–109.

Bos, R., Stojanova, A. S., Woerdenbag, H. J., Koulman, A., Quax, W. J. M. (**2005**)Volatile Components of the Aerial Parts of Artemisia pontica L.Grown in Bulgeria; *Flavour and Fragrance Journal*. Vol. 20.145-148.

Hammer, K. A., Carson, C. F, & Riley, T. V. (**1999**). Antimicrobial activity of essential oils and other plant extracts. *Journal of Applied Microbiolgy*, 86, 985–990.

Huang Y., Lam S.L., Ho S.H. (**2000**). Bioactivities of essential oil from *Elletaria cardamomum* (L.) Maton. To *Sitophilus zea- maise* Motschulsky and *Tribolium castaneum* (Herbst). *Journal of Stored Products Research*. 36 (2): 107–117.

Packard R. M. (**2007**).The Marking of a Tropical Disease: A Short History of Malaria. John Hopkins Biographers of Disease.

Plarre R., Poschko M., Prozell S., Frank A., Wohlgemuth R., Phillips J. K. (**1997**). Effects of oil of cloves and citronerol, two commercially available repellents, against the webbing clothes moth *Tineola bisselliella* Hum. (Lepidoptera: Tineidae). *Journal Pesticide Science* 70 (1): 45–50.

Regnault-Roger C., Hamraoui A. (**1995**). Fumigant toxic activity and reproductive inhibition induced by monoterpenes on *Aeanthoseelides obteetus* (Say) (Coleoptera), a bruchid of kidney bean *(Phaseolus vulgaris* L.). *Journal of Stored Products Research* 31 (4): 291–299.

Saxena C. R., Dixit P. D., Harshan V. (**1992**). Insecticidal action of *Lantana camara* against *Callosobruchus chinensis* (Coleoptera: Bruchidae). *Journal of Stored Products Research* 28 (4): 279–281.

Shaaya E., Kostjukovski M., Eilberg J.,

Sukprakarn C. (**1997**). Plant oils as fumigants and contact insecticides for the control of stored-product insects. *Journal Stored Products Research* 33 (1): 7–15.

Tabanca, N., Demirci, B., Blythe, E. K., Bernier, U. R., Ali, A., Wedge, D. E., Khan, I.A.; Bager, K.H.C. (**2011).** Composition of *Artemisia abrotanum* and *A. pontica* essential oils and their repellent activity against. *Aedes aegypti*. *Planta Med.*, *77*, PE52.

Wang J., Zhu F., Zhou X. M., Niu C.Y. and Lei C. I. (**2006**). Repellent and fumigant activityof essential oil from *Artemisia vulgaris* to *Tribolium eastaneum* (Herbst). *Journal of Stored Products Research* 42 (3): 339–347.

WHO (**1996**). Report of the WHO informal consultation on the evaluation and testing of insecticides. CTD/WHOPES/IC/96. 1. Control of Tropical Diseases Division. Geneva: WHO

Xie S.Y., Fields G.P., Isman B.M., Chen K.W., Zhang X. (**1995**). Insecticidal activity of *Melia toosendan* extracts and toosendanin against three stored products. *Journal of Stored Products Research* 31 (3): 259–265