AkiNik

American Journal of Essential Oils and Natural Products

Available online at www.essencejournal.com

AJEONP

American Journal of Essential Oils and Natural Products

ISSN: 2321-9114
AJEONP 2017; 5(3): 07-11

Received: 05-05-2017
Accepted: 06-06-2017

Basma NAJAR
Department of Pharmacy, University of Pisa, Via Bonanno Pisa, Italy

Luisa PISTELLI
Department of Pharmacy, University of Pisa, Via Bonanno Pisa, Italy

Correspondence
Basma NAJAR
Department of Pharmacy, University of Pisa, Via Bonanno Pisa, Italy

Essential Oil Composition of *Lawsonia inermis* leaves from Tunisia

Basma NAJAR and Luisa PISTELLI

Abstract
The composition of essential oil (EO) from *Lawsonia inermis* leaves collected in Gabes (South of Tunisia) were the object of this study. The EO was dominated by apocarotenoids (33.6%) followed by oxygenated sesquiterpenes (12.4%). Geranyl acetone was the main constituent of the EO with a percentage of 13.4%. β-ionene, responsible of violet-odor, occurred in relatively small amount (2.9%). Hierarchical Cluster Analysis (HCA) and Principal Compound Analysis (PCA) showed that Tunisian EO sample have a similar composition with those obtained from plants collected in Nigeria and Ethiopia while the Neger sample revealed a composition in itself.

Keywords: *Lawsonia inermis*, leaves, EO, HCA, PCA, South Tunisia

1. Introduction
Lawsonia inermis L., commonly known as Henné, belongs to the Lythraceae family. It is a plant native to North Africa and South-East Asia, and often cultivated as an ornamental and dye plant throughout India, Persia, and along the African coasts of the Mediterranean Sea [1]. This plant is known worldwide as cosmetic agent used to stain hair, skin and nails [2] and the oil obtained from its flower is used in perfumery [3]. *Lawsonia innermis* has also a potent pharmacological effect such as anti-tumor and antibacterial [4] antituberculosis [5], anti-parasitic [6], anti-inflammatory [7] and anti-trypanosomal [8]. Although previous study reported that various number of secondary metabolites were isolated from the non-volatile fraction [9-10], the essential oil composition was not studied in deep. The leaf essential oil contained other secondary metabolites such as 1, 8-cineole, α-pinene, and p-cymene [11]. Monoterpenes were also the main class of constituents of leaves emanated from Nepal [12]. Non-terpenes derivatives were the dominant class of composites, whose percentage oscillates between 40% in Ethiopia samples [13], to 53% in Ibadan (Nigeria) [14] to reach 78% in Malaysia [15].
The aim of this study was the essential oil analysis from *Lawsonia inermis* leaves cultivated in South Tunisia of and the analysis of its composition.

2. Methods and material
Air dried leaves of *L. inermis* were bought in Gabés (South Tunisia) and hydrodistillated using a Clevenger-type apparatus according to the Italian Pharmacopoeia [16]. Then the essential Oil (EO) was analyzed by GC-MS using Varian CP3800 gas chromatograph equipped with a DB-5 capillary column (30m x 0.25mm;coating thickness, 0.25 µm) and a Varian Saturn 2000 ion trap mass detector. Analytical condition was as follows: injector and transfer line temperature, 220° and 240 °C at 3 °C/min, respectively; oven temperature, programmed from 60 to 240 °C at 3 °C; carrier gas, helium at 1 mL/min; injection, 0.2 µL (10% hexane solution); split ratio, 1:30.
Identification of constituents was performed by comparison of their retention times (Rt) with those of pure reference samples andby means of their linear retention indices (LRIs) relative to the series of n-alkanes. The mass spectra were compared with those listed in the commercial libraries NIST 98 [17] and ADAMS [18], together with a home-made mass-spectral library, built up from pure substances and components of known oils, and with MS literature data. EO composition of collected plant was integrated with data available in literature from another provenance (Table 1). These data were used to build a matrix of all compounds which were present with a percentage ≥ 1%.

Statistical analysis: Past 3 software package' ver. 3.15 was used for statistical analysis. The hierarchical cluster analysis HCA was performed using Ward's method, with squared Euclidian distances as a measure of similarity.

3. Results and discussion

All the identified compounds of *L. inermis* essential oil were listed in Table 2.Totally, 80 compounds representing 94% of the whole volatile fraction were identified. Apocarotenoids were the main class of constituents identified (33.6%), followed by the non-terpene derivatives (19.8%), oxygenated sesquiterpenes (12.4%) and monoterpene hydrocarbons (9.9%) in the order. These results disagree with those reported in the literature in both quality and quantity. Henné leaf oil were dominated by ethyl hexadecanoate (24.4%), (*E*)-methyl cinnamate (11.4%), isocaryophyllene (8.1%), and (*E*)-α-ionone (5.8%) [14]. The abundance in Non-terpene derivatives class was also reported [13] where hexadecenoic acid was the main constituent of this class with 15%. The same behavior was noted henné EO from Malaysia [15] whose heptadecane was the most important constituent (23.5%) followed by tetradecane (16.8%) and hexadecane (14.9%). Phytol was present with the same percentage as in Ethiopia sample [13] as in Malaysia samples [15] (around 10%). Was also present in Ibadan (Nigeria) sample [14] in *iso*- format with percentage of 2.1%. Eugenol, although was the most important constituent of Afar (Ethiopia) sample (17.6%) [13], its presence crumbled to arrive at 0.6% in Ibadan (Nigeria) [14] sample and eventually disappear in Malaysia sample [15].

Country wise, A. Ogunbinu *et al* [11] noted that hénne essential oil was rich on monoterpenes 98.3% of whole composition where, 8-cineole (58.6%), α-pinene (18.1%) and *p*-cymene (14.7%) were the means constituents. This result was in agree with those of Satyal *et al*. [12] where the amount of monoterpenes exceed the 55% with limonene (20%), linalool (7.0%) and 1,8-cineol (6.9%) representing the mean composites of this class. The latter author [12], noted the abundance on phytol (27.5%) of hénne essential oil. The essential oil studied here was characterized by a higher percentage of geranylacetone (13.4%) and hexahydrofarnesyl acetone (11.5%) followed by hexadecanoic acid (8.3%) and farnesyl acetone (5.5%), which were not reported by earlier works.

The apocarotenoids which characterize Tunisian *Lawsonia* oil are organic compounds derived from carotenoids by oxidative cleavage (specific cleavage of the polyene chain double bonds) [19]. They are isoprenoid molecules which are important for primary and secondary metabolisms of plants [20]. The product of these oxidative cleavage can act as hormones (example, abscisic acid), signaling compounds (such as the visual and signaling molecules; retinal and retinoic acid), chromophores and scent/aroma constituents [21]. Among the aromatic volatilesβ-ionone was present with 2.9% in the studied Lawsonia EO. This compound contributed significantly to the fragrance [22], even if it was present in very low concentration in the scent [23]. Ionones differ slightly in their sensory properties: both α-ionone and β-ionone have a warm, woody, berry-characteristic violet-like odor, but β-ionone is more intense [24]. β-ionone was recognized by its strong inhibitor activity of the enzyme 3-hydroxy-3-methylglutaryl CoA reductase (HMGR), which is important for cholesterol biosynthesis and cell proliferation [25].This explains the observation done on the growth of breast cancer cells in vitro which were inhibited by β-ionone [26].

Hexahydrofarnesyl could have some defensive functions against fungi, because similar methyl ketones with long chains possess such activity [27].

Ishihara K *et al* [28] reported that hexadecanoic acid, a saturated fatty acid, suppressed eicosanoid production.

Integrating our results with data from the literature (Table 2), two different matrices were subjected to multivariate analysis. Hierarchical Cluster Analysis HCA (Fig.1) showed the presence of two main groups: one was represented by sample from Minna: Niger State (West of Nigeria) while the second one included all other provenances. This latter can be divided into two main subgroups: one with sample from Gabés and Asaita which were in correlation with Ibadan and by far with Biratnagar sample, and the second subgroup with Malaysia sample.

PCA were the first axis (PC1) explained for 51.0% and PC2 for 16.4%, which resumed 67.4% of the total variability (Fig.2), evidenced that the samples from Minna is characterized by 1,8-cineol and α-pinene. The subgroup from Malaysia showed heptadecane, tetradecane and hexadecane as well as phyto as constituent characteristics of this region. Phytol was also one of main constituent of sample from Biratnagar which explain its emplacement in the same part near Malaysia in PCA analysis. On the contrary, the presence of monoterpenes in the later region (Biratnagar) approaches it rather to the second subgroup of Gabés, Asayat and Ibadan samples. In addition, these samples are rich on non-terpenes derivatives which can also explain their presence in the lower left quadrat of the PCA analysis with percentage no less than 20%.

Table 1: Source of data and sampled locality for the phytochemical investigation

Locality	Country	Source of Data
Malaysia	Malaysia	Rahmat *et al*. 2006 [15]
Asayta	Ethiopia (Afar region)	Kidanemariam *et al*. 2013 [13]
Ibadan: Oyo state	Nigeria (South West)	O.Oyedeji and Ekundayo 2005 [14]
Minna: Niger State	Nigeria (West)	O.Ogunbinu *et al*. 2013
Biratnagar: Morangdistrict	Nipal (Koshi Zone)	Satyal *et al*. 2012 [12]
Gabés	Tunisia (South)	Presentstudy

Table 2: Chemical composition of EO of *Lawsonia inermis*

	Compounds	LRI	Class	%
1	(*E*)-2-hexanal	860	nt	0.2
2	tricyclene	938	mh	3.0
3	sabinene	978	mh	1.0
4	β-pinene	981	mh	0.1
5	6-mehty-5-hepten-2-one	990	nt	0.1
6	myrcene	993	mh	0.5
7	α-terpinene	1019	mh	0.4
8	*p*-cymene	1028	mh	0.4
9	limonene	1032	mh	1.0
10	(*E*)-β-ocimene	1053	mh	0.2
11	γ-terpinene	1062	mh	1.0
12	*p*-cymenene	1090	mh	0.3
13	terpinolene	1090	mh	0.6
14	linalool	1102	mh	1.2
15	N-nonanal	1104	nt	0.6
16	1,3,8-*p*-menthatriene	1110	mh	0.1
17	*cis*-*p*-mentha-2,8-dien-1-ol	1138	om	0.3
18	camphor	1148	om	0.2
19	*trans*-verbenol	1150	om	0.2
20	neroloxide	1158	om	0.1
21	(*E*)-2-nonen-1-al	1164	nt	0.2
22	terpinen-4-ol	1180	om	0.3
23	safranal	1200	ac	0.3
24	N-decanal	1206	nt	0.3
25	*trans*-pulegol	1215	om	1.0
26	*trans*-carveol	1221	om	0.3
27	*cis*-pulegol	1229	om	0.1
28	ascaridole	1237	om	0.2
29	*iso*bornylacetate	1287	om	0.6
30	carvacrol	1301	om	0.7
31	nerylacetate	1368	om	1.6
32	α-copaene	1376	sh	0.1
33	longifolene	1404	sh	0.1
34	dodecanal	1409	nt	0.2
35	β-cariophyllene	1418	sh	0.9
36	*cis*-dictamol	1430	nt	0.2
37	α-guaiene	1440	sh	0.3
38	geranylacetone	1455	ac	13.4
39	γ-muurolene	1477	sh	0.2
40	germacrene D	1481	sh	0.6
41	(*E*)-β-ionene	1485	ac	2.9
42	*cis*-β-guaiene	1489	sh	0.5
43	α-muurolene	1499	sh	1.5
44	(*E*-*E*)-α-farnesene	1507	sh	0.9
45	*trans*-γ-cadinene	1513	sh	1.2
46	δ-cadinene	1523	sh	1.5
47	β-thujaplicinol	1532	nt	3.3
48	α-cadinene	1537	sh	0.3
49	(*E*)-nerolidol	1566	os	1.4
50	globulol	1584	os	0,3
51	thujapsan-2-α-ol	1587	os	1.1
52	cartol	1595	os	0.4
53	guaiol	1597	os	0.7
54	5-*epi*-7-α-eudesmol	1606	os	2.2
55	α-acorenol	1633	os	0.7
56	β-acorenol	1634	os	0.3
57	*epi*-α-cadinol	1642	os	1.2
58	α-muurolol	1651	os	0.2
59	α-cadinol	1655	os	0.7
60	intermediol	1667	os	0.2
61	N-tetradecanol	1675	nt	0.5
62	β-bisabolol	1675	os	0.2
63	elemolacetate	1681	nt	0.5
64	α-bisabolol	1684	os	0.2
65	hetadecane	1700	nt	0.4

66	(*Z*-*E*)-farnesol	1701	os	0.1
67	(*E*-*E*)-farnesol	1719	os	0.5
68	tetranoicacid	1765	nt	3.1
69	(*Z*-*E*)-farnesylacetate>	1824	os	0.2
70	khusinolacetate	1827	nt	0.4
71	cyclopentadecanolide	1836	os	0.2
72	(*E*-*E*)-farnesylacetate	1843	os	1.5
73	Hexahydrofarnesylacetone	1845	ac	11.5
74	farnesylacetone	1920	ac	5.5
75	methylhexadecanoate	1931	nt	1.3
76	phytol	1950	od	2.0
77	hexadecanoicacid	1977	nt	8.3
78	manoyloxide	1987	od	1.0
79	abietatriene	2055	dh	0.5
80	abietadiene	2079	dh	1.0
	Monoterpenehydrocarbons (mh)			9.8
	Oxygenatedmonoterpenes (om)			5.6
	Sesquiterpene hydrocarbones (sh)			8.2
	Oxygenatedsesquiterpenes (os)			12.4
	Diterpenehydrocarbons (dh)			1.6
	Oxygenatedditerpenes (od)			3.0
	Apocarotenoides (ac)			33.6
	Non-terpenederivatives (nt)			19.8
	Total			94.0

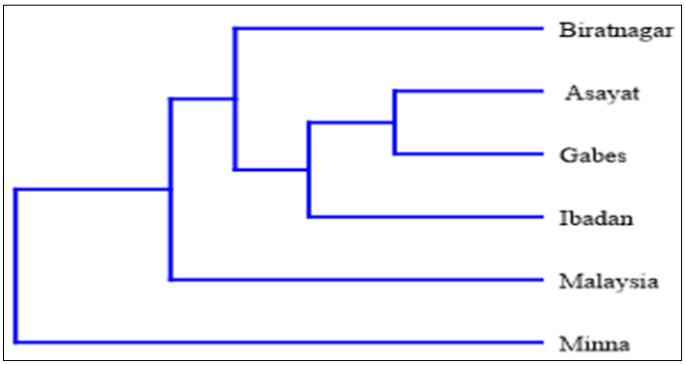

Fig 1: Dendrogram of the Hierarchical Cluster Analysis(HCA) of different countries.

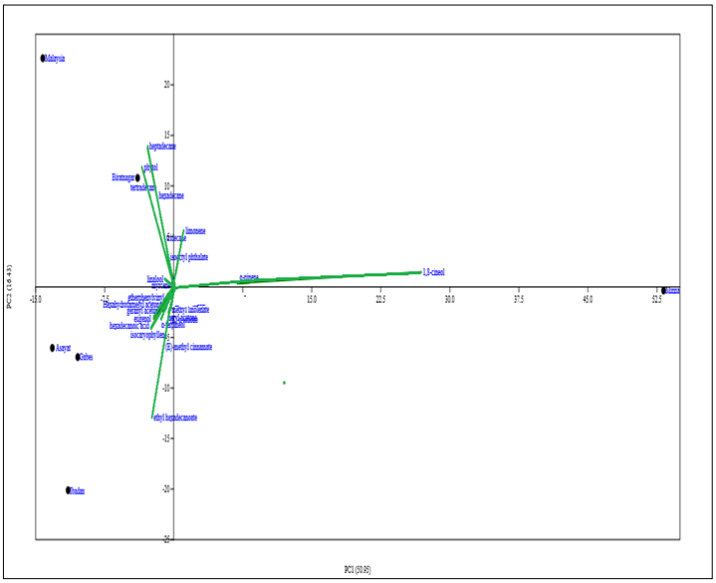

Fig 2: Principal Compound Analysis (PCA) plot of mean compounds of EOs of samples from different countries.

4. References

1. Malekzadeh F. Antimicrobial Activity of *Lawsonia inermis* L. Applied microbial. 1968; 16(4):663-664.
2. Rostkowska H, Nowak MJ, Lapinski L, Adamowicz L. Molecular structure and infrared spectra of 2-hydroxy-1, 4- naphthoquinone; Experimental matrix isolation and theoretical Hartree–Fock and post Hartree-Fock study. Spec. Act. 1998; 54(8):1091-1103.

3. Jain SK. Henna. In: Medicinal plants. National Book Trust India. 1999; 112-3.
4. Ali NA, Jülich WD, Kusnick C, Lindequist U. Screening of Yemeni medicinal plants for antibacterial and cytotoxic activities. J Ethnopharmacol. 2001; 74(2):173-179.
5. Sharma VK. Tuberculostatic activity of henna (*Lawsonia inermis* Linn.). Tubercolosis. 1990; 71(4):293-295.
6. Okpekon T, Yolou S, Gleye C, Roblot F, Loiseau P, Bories C *et al*. Antiparasitic activities of medicinal plants used in Ivory Coast. J.Ethnopharm. 2004; 90(1):91-97.
7. Yogisha S, Samiulla DS, Prashanth D, Padmaja R, Amit A. Trypsininhibitory activity of *Lawsonia inermis*. Fitoterapia. 2002; 73(7-8):690-691.
8. Atawodi SE, Ameh DA, Ibrahim S, Andrew JN, Nzelibe HC, Onyike EO *et al*. Indigenous knowledge system for treatment of trypanosomiasis in Kaduna state of Nigeria. J. Ethnopharmacol. 2002; 79(2):279-282.
9. Handa G, Kapil A, Sharma S, Singh J. Lawnermis acid: a new anticomplementary triterpenoid from *Lawsonia inermis* seeds. Indian J. Chem., Sect. B 1997; 36:252-256.
10. Alam MS, Niwa M, Sakai T, Gupta S, Ali M. 24β-Ethylcholest-4-en-3β-ol from the roots of *Lawsonia inermis*. Phytochemistry. 1992; 31(7):2558-2560.
11. Ogunbinu AO, Ogunwande IA, Walker TM, Setzer WN. Study on the Essential Oil of *Lawsonia inermis* (L) Lythraceae. Jeob. 2007; 10(3):184-188.
12. Satyal P, Paudel P, Poudel A, Setzer WN. Antimicrobial activities and constituents of the leaf essential oil of *Lawsonia inermis* groing in Nepal. Pharmacology OnLine, 2012; 1:31-35.
13. Kidanemariam TK, Tesama TK, Asressu KH, Boru AD. Chemical Investigation of *Lawsonia inermis* L. Leaves from Afar Region, Ethiopia. Oriental J Chem, 2013; 29(3):1129-1134.
14. Oyedeji OA, Ekundayo O, Koenig WA. Essential oil composition of *Lawsonia inermis* L. leaves from Nigeria. J. Essent. Oil Res. 2005; 17:403-404.
15. Rahmat A, Edrini S, Ismail P, Yap Yun Hin T, Abu Bakar MF. Chemical Constituents, antioxidant activity and cytotoxic effects of essential oil from *Stobilan thescrispus* and *Lawsonia innernis*. J. Biol Sci. 2006, 6(6):1005-1010
16. AOAC. Official Methods of Analysis. Helrich. K. (Ed) 15th Edition. AOAC. Arlington. VIRGINIA. 1990, 1298.
17. Stein S, Mirokhin D, Tchekhovskoi D, Mallard G. Data Evalution by Mikaia A, Neta P, Sparkman D, White E, Yang X, Zaikin V, Zhu D. The NIST Mass Spectral Search Program for the NIST/EPA/NIH Mass Spectral Library. Version 2.0g. Build, 2014.
18. Dams RPA. Identification of essential oil composition by gas chromatography/ quadrupole mass spectroscopy. Allured Carol Stream, IL 60188, USA ISBN 0-931710-85-5, 2001.
19. Marasco EK, Vay K, Schmidt-Dannert C. Identification of Carotenoid Cleavage Dioxygenases from Nostocsp. PCC 7120 with Different Cleavage Activities. J Biol Chem (ASBMB). 2006; 281(42):31583-31593.
20. Carlo R, Gianfranco D, Giovanni G. Biosynthesis and Engineering of Carotenoids and Apocarotenoids in Plants: State of the Art and Future Prospects. Biotechnol Genet Eng Reviews. 2009; 26:151-174.
21. Walter MH, Floss DS, Strack D. Apocarotenoids: hormones, mycorrhizal metabolites and aroma volatiles. Planta. 2010; 232(1):1-17.
22. Maia AC, Gibernau M, Dotterl S, Navarro DM, Seifert K, Mŭller T *et al*. The floral scent of *Taccarumulei* (Araceae): attraction of scarab beetle pollinators to an unusual aliphatic acyloin. Phytochemistry. 2013; 93:71-8.
23. Nath P, Bouzayen M, Pech JC, Mattoo AK. Fruit ripening: physiology, signalling and genomics. Oxfordshire: CABI, 2014.
24. Burdock GA. Fenaroli's Handbook of Flavour Ingredients. (4th ed.), CRC Press, Boca Raton, 2001.
25. Mo HB, Elson CE. Apoptosis and cell-cycle arrest in human and murine tumor cells are initiated by isoprenoids. J N. 1999; 129:804-813.
26. Duncan RE, Lau D, El-Sohemy A, Archer MC. Geraniol and beta-ionone inhibit proliferation, cell cycle progression, and cyclin-dependent kinase 2 activity in MCF-7 breast cancer cells independent of effects on HMG-CoA reductase activity. Biochem Pharmacol. 2004; 68:1739-1747.
27. Platikanova S, Nikolovb S, Pavlovac D, Evstatievaa L, Popovd S. Volatiles from Four Astragalus Species: Phenological Changes and their Chemotaxonomical Application. *Z.* Naturforsch. 2005; 60c:591D599.
28. Ishihara K, Murata M, Kaneniwa M, Saito H, Shinohara K, Maeda-Yamamoto M. Inhibition of icosanoid production in MC ⁄ 9 mouse mast cells by n-3 polyunsaturated fatty acids isolated from edible marine algae. Biosci Biotechnol Biochem. 1998; 62:1412-1415.