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Acetylcholinesterase Inhibitory Effect and Characterization of the Essential Oil of *Plectranthus aegyptiacus* (Forssk.) C. Chr. Growing in Nigeria

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Abstract

Essential oil of fresh leaves of *Plectranthus aegyptiacus* (Forssk.) C. Chr. collected in Nigeria was analyzed to determine its chemotype. A total of 30 compounds were identified. Constituents identified were mainly mono and sesquiterpenoids with the major components, accounting for about 76% of the components, being α-terpinene (6.9%), p-cymene (14.2%), γ-terpinene (5.5%), carvacrol (10.0%), α-copaene (5.3%), α-caryophyllene (5.8%), germacrene-D (11.6%), δ-cadinene (8.4%) and α-cadinol (8.4%). The essential oil showed anticholinesterase inhibitory activity with an IC_{50} of 8.29 ± 0.67 mg/ml.

Keywords: *Plectranthus aegyptiacus*; Essential oil composition; p-cymene; Carvacrol; Germacrene-D; Anticholinesterase

Introduction

In continuation of our interest in the essential oils of the family Lamiaceae [1], we were particularly attracted to the variety of *Plectranthus aegyptiacus* (Forssk.) C. Chr. growing in Nigeria as an ornamental plant because of its remarkable resemblance, in odour, to the *Ocimum gratissimum* variety (thymol chemotype) available in the country. Literature indicates three distinct chemotypes of *P. aegyptiacus*, synonym *P. tenuiflorus* (Vatke) Agnew, differing in their essential oil compositions. Thymol is the dominant component of the essential oil of leaves harvested from the Taif West Highlands of Saudi Arabia, with wound healing property [2]. However, the leaf harvested from Abha South highlands of the same country is composed majorly of δ-3-carene (52.8%) [3]. On the other hand, carvacrol (14.3%), p-cymene (10.9%) and α-terpinene (10.2%) were reported as the major components of the leaves collected from Nairobi, Kenya [4].

The essential oils of several plants and their components have been reported to possess anticholinesterase activities [5-8]. The potential of essential oil constituents (either as single compounds or as mixtures) to inhibit acetylcholinesterase (AChE) have been attributed to their small molecular size and lipophilic nature.

Materials and Methods

Chemicals and reagents

Acetylthiocholine (ATCI) and 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) were purchased from Sigma-Aldrich Chemicals (St. Louis, Missouri, USA).

Enzyme preparation

The enzyme was prepared by homogenizing rat brain in phosphate buffer (50 mM, pH 7.4). The mixture was centrifuged using a cold centrifuge. The supernatant was collected and store in a freezer (-20°C) until ready for use as previously reported [9].

Plant material

P. aegyptiacus was collected from the cultivation established at the staff quarters, Obafemi Awolowo University campus. The plant was identified and authenticated at KEW Gardens, London and a voucher specimen has been deposited at the Forestry Herbarium, Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria as FHI. 111187. Leaves were collected in June, at the rainy season, during which period the plant flourishes.

Extraction of the fresh leaves

Fresh leaf materials were pulverised and hydro-distilled in Clevenger-type apparatus for 4 hr to obtain the volatile oil. The oil samples were stored in vials and kept in freezer (-4°C) until needed. Repeated collections were separately hydro-distilled and analysed by GC and GC-MS.

Gas chromatography-mass spectrometry analysis

The composition of the essential oils obtained was determined by GC and GC-MS analysis. The oil was analysed by GC-MS (FOCUS-ISQ, Thermoscientific) in the electron ionisation mode operating at 70 ev and separation achieved on a ZB-5ms capillary column (30 m × 0.25 mm ID × 0.25 µm film thickness) (Phenomenex). Temperature program: from 40°C (2 min) to 200°C (10 min) at 5°C/min). The relative compositions of the essential oils were determined on Agilent 7890B coupled to FID and an autoinjector (Agilent G45138). Column: 19091J-413 HP-5 (5% phenyl methyl siloxane) 30 m × 0.32 mm ID × 0.25 µm film thickness (Agilent). Temperature program: from 50°C (5 min) to 200°C (10 min) at 2°C/min. Injection temperature: 250°C. Injection volume: 1.0 µL. Inlet pressure: 66.7 kPa. Carrier gas: He, Linear velocity: 40 cm/sec. Injection mode: split (50:1). FID temp.: 230°C; H_2 flow: 40 mL/min; air flow: 400 mL/min. The oil sample was dissolved in dichloromethane before injection. The eluted compounds were identified by comparing with authentic monoterpene standards, determining Kovat's indices (KI) using n-alkane standards, and by mass spectral matching with NIST library.

Cholinesterase inhibitory assay

AChE inhibitions were determined spectrophotometrically using ATCI as substrate, by the modified method of Ellman et al. [10] as described by Giovanni et al. [11]. In 96-well plates was added 240 µl of buffer (50 mM Tris-HCl, pH 8.0), 20 µl of varying concentrations (10, 5, 2.5 and 1.25 mg/ml) of the essential oil test samples dissolved in 5% dimethyl sulphoxide and 20 µl of the enzyme preparation. The reaction mixture was then incubated for 30 min at 37°C, after which 20 µl of 10 mM DTNB was added. The reaction was then initiated by the addition of 20 µl of 25 mM ATCI. The rate of hydrolysis of ATCI was then determined spectrophotometrically by measuring the change in the absorbance per minute (ΔA/min) due to the formation of the yellow 5-thio-2-nitrobenzoate anion at 412 nm over a period of 4 min at 30 s intervals. A solution of buffer was used as negative control. All assays were carried out in triplicate. Eserine was used as positive control.

The percentage inhibition (%I) of test sample was obtained using the formula:

$$I\ (\%)=[(V_o-V_i)/\ V_o] \times 100,$$

Where: I (%)=Percentage inhibition,

V_i=enzyme activity in the presence of test sample,

V_o=enzyme activity in the absence of test sample.

The IC_{50} was obtained by plotting a linear regression curve of the percentage inhibition (%) of the enzymes against essential oils concentration. Regression equation was used in computing the IC_{50} values.

Results and Discussion

The hydro-distilled fresh leaf material of *P. aegyptiacus* collected during the rainy season gave pale yellow oil (0.15% v/m, ρ=0.81 g/ml).

A total of 30 compounds accounting for approximately 94% oil content were identified (Table 1; Figure 1).

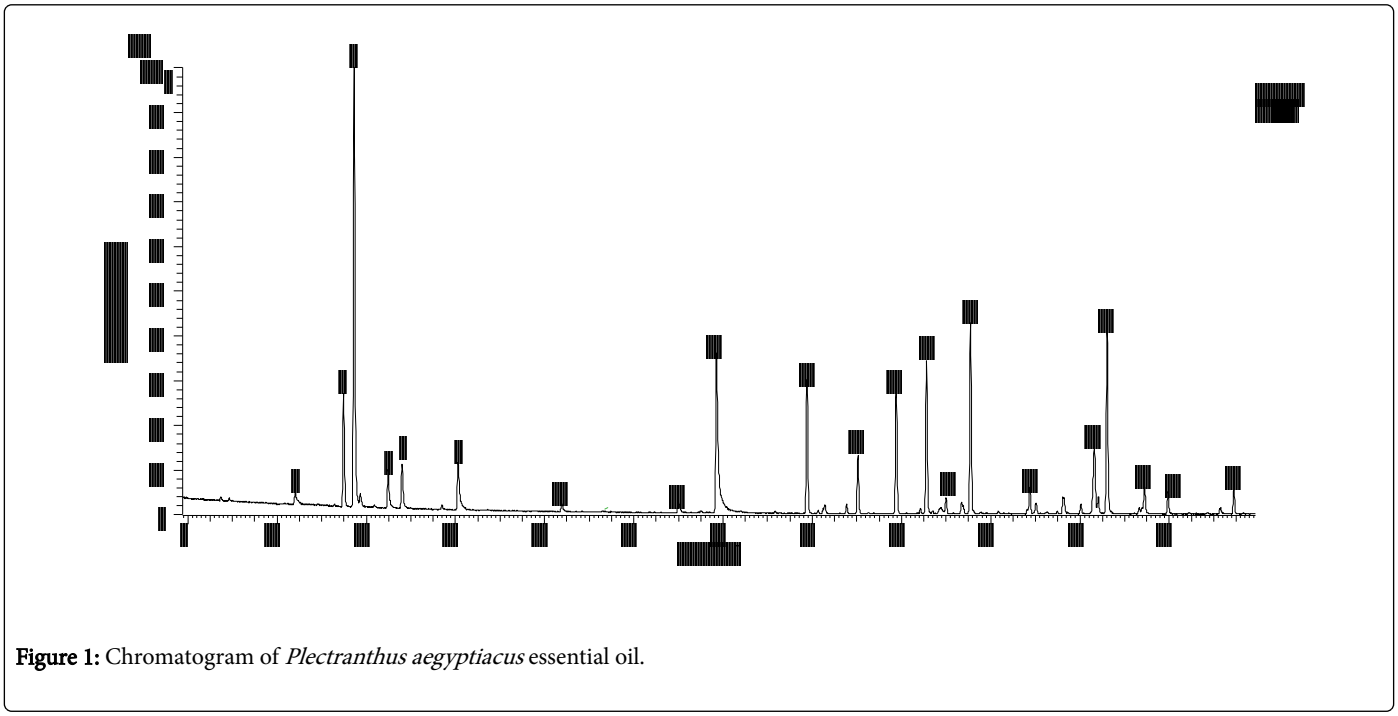

Figure 1: Chromatogram of *Plectranthus aegyptiacus* essential oil.

The major compounds were identified as α-terpinene (6.9%), p-cymene (14.2%), γ-terpinene (5.5%), carvacrol (10.0%), α-copaene (5.3%), α-caryophyllene (5.8%), germacrene-D (11.6%), δ-cadinene (8.4%) and α-cadinol (8.4%). which constituted 76.2% of the total oil. The composition of the compounds was determined as an average of determinations from two different plant collections (>5% of the total essential oil of the wet leaf). Other significant components which characterised the essential oil are β-ocimene (E) (2.7%), linalool (2.2%), β-caryophyllene (2.6%) and α-muurolol (3.7%). In addition, four high boiling sesquiterpenes were detected in the essential oils but could not be completely identified.

S/No	Compound	KI	Composition (%)
1.	1-octen-3-ol	980	1.0
2.	α–phellandrene	1004	0.3
3.	α–terpinene	1016	6.9
4.	p-cymene	1024	14.2
5.	β-phellandrene	1029	0.4
6.	β -ocimene (Z)	1039	0.1
7.	β -ocimene (E)	1049	2.7
8.	γ-terpinene	1060	5.5
9.	Linalool	1100	2.2
10.	terpinen-4-ol	1179	0.3
11.	unknown	1273	0.4
12.	Carvacrol	1303	10.0
13.	α–copaene	1380	5.3
14.	β–elemene	1395	0.4
15.	α–gurjunene	1415	0.2
16.	β–caryophyllene	1425	2.6
17.	α–caryophyllene	1460	5.8
18.	g-muurolene	1482	0.1
19.	germacrene-D	1487	11.6
20.	β-Guaiene, trans	1493	trace
21.	unknown	1500	0.2
22.	germacrene-B	1503	0.8
23.	α–muurolene	1505	0.3
24.	γ-cadinene	1520	0.4
25.	δ-cadinene	1528	8.4
26.	Germacrene D-4-ol	1582	0.4
27.	Spathulenol	1583	0.5
28.	Unknown	1591	0.3
29.	γ-cadinol	1618	0.5
30.	τ-cadinol	1635	0.3
31.	α-muurolol	1649	3.7
32.	τ-muurolol	1652	0.4
33.	α–cadinol	1661	8.4
34.	Unknown 204(6%), 161(40%), 105(50%), 84(100%), 81 (87%), 41(70%)	1699	2.3
35.	Unknown	1724	1.0

	M+218(5%), 203(10%), 176(27%), 161(100%), 147(25%), 124(62%), 105(35%), 95(42%), 41(52%)		
36.	Unknown 202(12%), 187(24%), 174(6%), 159(100%)	1793	1.1
	Total composition (%)		**99**

Table 1: Essential oil composition of fresh leaves of *Plectranthus aegyptiacus* as mean of two collections.

In spite of its remarkable odoriferous resemblance to *O. gratissimum*, which contains thymol as the predominant compound [1], the leaves of *P. aegyptiacus* that we investigated showed absence of thymol. On the other hand, carvacrol, known to resemble thymol in odour [12] is one of the major components of the sample of *P. aegyptiacus*. The collection closely resembles the Kenya sample [4] with the three most prominent monoterpenes being α-terpinene, p-cymene, and carvacrol. However, there are qualitative differences in the sesquiterpenes and this may have arisen from the differences in processing. The collections reported here were distilled as fresh materials while the Kenya sample reported in literature was semi-dried. The three most prominent sesquiterpenes are δ-cadinene, Germacrene D and α-cadinol. The present collection is clearly different from the Saudi chemotypes reported in literature.

Although α-pinene and carvacrol had been previously reported to possess anticholinesterase activities [6,7], the anticholinesterase activity of the present material has not been attributed to any component in particular as the literature has demonstrated remarkable synergism with combination of lower terpenes which are otherwise inactive as single components and antagonism between compounds which are individually active (Table 2) [8].

PA		Eserine		IC_{50} (mg/ml)
Concentration (mg/ml)	**Mean% Inhibition ± SEM**	**Concentration (mg/ml)**	**Mean% Inhibition ± SEM**	
10	55.37 ± 1.48	1	82.64 ± 2.69	PA=8.29 ± 0.67
5	39.52 ± 4.21	0.5	78.01 ± 1.68	
2.5	33.24 ± 1.64	0.25	73.45 ± 3.23	Eserine=0.14 ± 0.02
1.25	31.40 ± 1.31	0.125	65.79 ± 4.20	
0.625	23.91 ± 2.86	0.0625	61.29 ± 5.77	
0.3125	3.14 ± 5.94	0.03125	53.50 ± 8.57	

Table 2: Anticholinesterase activity of *Plectranthus aegyptiacus* leaf essential oil (PA) with eserine as standard.

Conclusion

The major components of the essential oil of *Plectranthus aegyptiacus* leaves are α-terpinene, p-cymene, γ-terpinene, carvacrol, α-copaene, α-caryophyllene, germacrene-D, δ-cadinene and α-cadinol. The oil showed moderate anticholinesterase inhibitory activity with an IC_{50} of 8.29 ± 0.67 mg/ml.

Declaration of Interest

The authors declare that they have no conflict of interest to disclose.

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References

1. Olugbade TA, Kolipha-Kamara MI, Elusiyan CA, Onawunmi GO, Ogundaini AO (2017) Essential oil chemotypes of three Ocimum species found in Sierra Leone and Nigeria. Medicinal and Aromatic Plants 6: 284.
2. Smith RM, Albar HA, Bahaffi SO (1996) Chemical composition of the essential oil of Plectranthus tenuiflorus from Saudi Arabia. Journal of Essential Oil Res 8: 447-448.
3. Al-Yahya MA, Hifnawy MS, Mossa JS, Al-Meshal IA (1985) Aromatic plants of Saudi Arabia, part 7: Essential oil of Plectranthus tenuiflorus (Vatke) Agnew. Proc Saudi Bio Soc 8: 47-153.
4. Mwangi JW, Lawande W, Hassanali A (1993) Composition of essential oil of Plectranthus tenuiflorus (Vatke) Agnew. Flavour and Fragrance Journal 8: 51-52.
5. Dohi S, Terasaki M, Makino M (2009) Acetylcholinesterase inhibitory activity and chemical composition of commercial essential oils. Journal of Agricultural and Food Chemistry 57: 4313-4318.
6. Aazza S, Lyoussi B, Miguel MG (2011) Antioxidant and antiacetylcholinesterase activities of some commercial essential oils and their major compounds. Molecules 16: 7672-7690.
7. Askina H, Yildiza M, Ayar A (2017) Effects of thymol and carvacrol on acetylcholinesterase from Drosophila melanogaster. Acta Physica Polonica A 132: 720-722.
8. Kitphati W, Wattanakamolkul K, Lomarat P, Phanthong P, Anantachoke N, et al. (2012) Anticholinesterase of essential oils and their constituents from Thai medicinal plants on purified and cellular enzymes. Journal of Asian Association of Schools of Pharmacy 1: 58-67.
9. Konrath EL, Neves BM, Lunardi PS, Passos CS, Simões-Pires A, et al. (2012) Investigation of the in vitro and ex vivo acetylcholinesterase and antioxidant activities of traditionally used Lycopodium species from South America on alkaloid extracts. Journal of Ethnopharmacology 139: 58-67.
10. Ellman GL, Courtney KD, Featherstone RM (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. Biochemical Pharmacology 7: 88-95.
11. Giovanni SD, Borloz A, Reist M (2008) In-vitro screening assays to identify natural or synthetic acetylcholinesterase inhibitors: Thin layer

chromatography versus microplate methods. European Journal of Pharmaceutical Science 33: 109-119.

12. Merck (1976), The Merck Index. 9th edn. Merck and Co. Inc., Rahway, New Jersey.