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 2014; 2 (1): 63-66

Received: 24-06-2014
Accepted: 23-08-2014

Oladipupo A. Lawal
Natural Product Research Unit Department of Chemistry, Faculty of Science Lagos State University, PMB 0001 LASU Post Office Ojo, Lagos, Nigeria.

Isiaka A. Ogunwande
Natural Product Research Unit Department of Chemistry, Faculty of Science Lagos State University, PMB 0001 LASU Post Office Ojo, Lagos, Nigeria

Olusola F. Olorunloba
Natural Product Research Unit Department of Chemistry, Faculty of Science Lagos State University, PMB 0001 LASU Post Office Ojo, Lagos, Nigeria.

Andy R. Opoku
Department of Biochemistry and Microbiology, University Zululand, KwaDlangezwa 3886 South Africa.

Correspondence:
Oladipupo A. Level
Natural Products Research Unit, Department of Chemistry, Faculty of Science, Lagos State University, PMB 0001 LASU Post Office, Ojo, Lagos, Nigeria.
Email: jumobi.lawal@lasu.edu.ng

The essential oils of *Chrysanthemum morifolium* Ramat. from Nigeria

Oladipupo A. Lawal, Isiaka A. Ogunwande, Olusola F. Olorunloba and Andy R. Opoku

Abstract
The essential oils obtained by hydrodistillation from the flowers and leaves of *Chrysanthemum morifolium* Ramat. were analyzed by gas chromatography (GC) and gas chromatography coupled with mass spectrometry (GC/MS). Thirteen and twenty-one components representing 97.5% and 93.7% of the flower and leaf oil contents were identified respectively. The major constituents of flower oil were *cis*-chrysantheny acetate (21.6%), octadecanoic acid (19.5%) and borneol (15.5%). However, the leaf oil was characterized by borneol (20.5%), 1,8-cineole (15.2%), *trans*-α-bergamotene (14.0%), camphor (10.6%) and α-pinene (9.3%). This is the first report on the chemical composition of essential oils of *Chrysanthemum morifolium* from Nigeria.

Keywords: Chrysanthemum morifolium, essential oil composition, cis-chrysantheny acetate, octadecanoic acid, *trans*-α-bergamotene, camphor.

1. Introduction
Chrysanthemum morifolium Ramat (Asteraceae) with a high ornamental value is one of the most popular traditional flowers and one of the most popular cut flowers in the world. It therefore occupies a very important position in the world flower industry [1]. The flowers of *C. morifolium* have been used in Vietnam and other Asian countries for the treatment of eye diseases, headaches, insomnia, and hyperglycemia. Extracts of *C. morifolium* have antioxidant [2-4], cardiovascular protective and anti-inflammatory functions [5] and potent neuroprotective activity and therefore, might be a potential candidate in neurodegenerative diseases such as Parkinson's disease [6, 7]. Polyphenols from *C. morifolium* extract attenuates high-fat milk-induced fatty liver through peroxisome proliferator-activated receptor α-mediated mechanism in mice [8]. An endoperoxysesquiterpene lactone, 10α-hydroxy-1α,4α-endoperoxy-guaia-2-en-12,6α-olide showed strong inhibitory effects against α-glucosidase and lipase activities [9] while the flavone glycosides, acacetin-7-*O*-β-d-glucopyranoside and acacetin-7-*O*-α-l-rhamopyranoside inhibited both α-glucosidase and α-amylase and eriodictyol was only effective against α-amylase [9]. Aqueous extract of flowers of the *C. morifolium* afforded arabinogalactan [10], phenolic compounds and caffeoylquinic acids [11]. Luteolin-7-*O*-β-glucuronide isolated from *C. morifolium* possesses allelopathic activity [12]. *N*-isobutyl-6-(2-thienyl)-2*E*,4*E*-hexadienamide, a nitrogenous compound isolated from the plant could act as numbing principle [13]. In addition, the sesquiterpenes, chrysanthediol A, chrysanthediacetate B and chrysanthediacetate C and β-dictyopterol [14], chrysandiol, chrysartemins A and B 1 and chlorochrymorin [15] were also characterized from *C. morifolium*. Some flavonoids were also present in the plant [16-18]. They include luteolin and diosmetin which have displayed cytotoxic activity [20], acacetin, apigenin, luteonin and quercetin that exhibited antimutagenic potential [21]. Other compounds were known as antibacterial, antifungal, antiviral, antispirochetal [22] and anti-HIV agents [23]. Chlorogenic acid (5-*O*-caffeoylquinic acid), 3,5-*O*-dicaffeoylquinic acid and 3,4,5-trihydroxyflavanone 7-*O*-glucuronide (eriodictyol 7-*O*-glucuronide) characterized from *C. morifolium* have shown phytotoxic and insect growth regulating activity [24]. Triterpenoids with antimicrobial [25], strong anti-inflammatory [26], anti-tumor and cytotoxic [27] effects were also isolated from *C. morifolium*.
Literature information has shown that few reports are available on compositions of essential oils from the flowers of *C. morifolium*. The main constituents of the previously studied oils

were mainly monoterpenes and sesquiterpenes such as β-caryophyllene (16.3%) and ledene oxide I (9.0%) [19], β-elemene camphor and borneol [28], camphor (27.14%) and bisabolol oxide (20.87%) [29, 31], juniper camphor [30], verbene oxides (25.32%) and chrysanthenone (8.26%) [31], 1,8-cineole (21.33%) [31], β-selinene (17.85%) and borneol (12.84%) [31], verbenyl acetate (32.10%) and α-curcumene (8.28%) [31], chrysanthenyl acetate (43.74%) and verbenol (27.85%) [32], 1,8-cineole, camphor and borneol [33].

In this paper, the chemical compounds of essential oils obtained from the flower and leaf of *C. morifolium* were being reported, as part of an extensive research on the chemical analysis of poorly studied species of Nigeria flora [34].

2. Materials and methods

2.1 Plant material

Fresh plant materials of *C. morifolium* were purchased from private garden in Surulere Area of Lagos State, Nigeria. Identification of the plant material was carried out at the Department of Botany, University of Lagos, Akoka-Yaba, Lagos, Nigeria. Specimen number (LUH 6731) was deposited in the Herbarium of the University.

2.2 Extraction of the volatile oils

Fresh flowers (35 g) and leaves (100 g) of *C. morifolium* were separately subjected to hydrodistillation for 3 h by means of Clevenger-type apparatus in accordance with the British Pharmacopoeia Specification [35]. The distillate isolated was preserved in a sealed sample tube and stored under refrigeration until analysis.

2.3 Analysis of the oil samples

GC analyses of the oils were carried out on a Hewlett Packard HP 6820 Gas Chromatograph equipped with a FID detector and DB-5 column (60 m x 0.25 mm id), film thickness was 0.25 µm and the split ratio was 1:25. The oven temperature was programmed from 50 °C (after 2 min) to 240 °C at 5 °C/min and the final temperature was held for 10 min. Injection and detector temperatures were 200 °C and 240 °C, respectively. Hydrogen was the carrier gas. An aliquot (0.5 µL of the diluted oil) was injected into the GC. Peaks were measured by electronic integration. A homologous series of *n*-alkanes were run under the same conditions for determination of retention indices.

GC-MS analyses of the oils were performed on a Hewlett Packard Gas Chromatograph HP 6890 interfaced with a Hewlett Packard 5973 mass spectrometer system equipped with a DB-5 capillary column (30 m x 0.25 mm id, film thickness 0.25 µm) under the same conditions the GC column. The oven temperature was programmed from 70-240 °C at the rate of 5 °C/min. The ion source was set at 240 °C and electron ionization at 70 eV. Helium was used as the carrier gas at a flow rate of 1 mL/min. The scanning range was 35 to 425 amu. Diluted oil in *n*-hexane (1.0 µL) was injected into the GC/MS.

2.4 Identification of compounds

The components of the oils were identified base on the comparison of their retention indices and mass spectra with those standards, Wiley library mass spectra database of the GC/MS system and published data [36, 37].

3. Results and Discussion

Table 1 displayed the identities and percentage composition of the constituents present in both oil samples. Thirteen compounds amounting to 97.5% of the total oil contents were identified in the flower oil. Oxygen containing monoterpenes (54.5%), their hydrocarbon counterparts (17.9%) and fatty acids (25.1%) represent the classes of compounds identified in the oil. Sesquiterpene compounds were conspicuously absent contrary to previous reports [19, 28, 29, 31]. The major constituents of the oil were *cis*-chrysantheny acetate (21.6%), octadecanoic acid (19.5%) and borneol (15.5%). There were significant quantity of camphor (8.7%), α-pinene (8.3%), camphene (6.1%) and 1,8-cineole (6.7%). The high content of fatty acid is unusual and noteworthy as this class of compound has not been reported to be of significant amount in *C. morifolium* oils.

Oxygenated monoterpenes (48.5%), monoterpene hydrocarbons (23.5%) and sesquiterpene hydrocarbons (17.5) were the main classs of compound present in the leaf oil. However, the oil was characterized by quantitative amount of borneol (20.5%), 1,8-cineole (15.2%), *trans*-α-bergamotene (14.0%), camphor (10.6%) and α-pinene (9.3%). Previous analysis on the essential oils of *C. morifolium* have focused on the flower. The present study on the leaf oil may be the first of its kind.

Comparing the results with previously analysed data, notable compounds such as ledene oxide I [19], β-elemene [28], bisabolol oxide [29, 33], verbene oxides, chrysanthenone, β-selinene, verbenyl acetate, α-curcumene [31], verbenol [32] which are characteristics of said reports were conspicuously absent in the presently investigated oil samples. In addition, it could be noted that octadecanoic acid and *trans*-α-bergamotene were not mentioned to be of qualitative and quantitative importance in previous analyses. The contents of camphor and β-caryophyllene are low when compared with previous data.

3.1 Tables

Table 1: Chemical composition of *Chrysanthemum morifolium* essential oils

Compounds [a]	RI[b]	RI [c]	Percent composition	
			Flower	Leaf
α-Pinene	937	932	8.3	9.3
Camphene	952	946	6.1	4.3
Sabinene	975	969	1.7	-
β-Pinene	978	974	0.7	1.4
β-Phellandrene	1001	1002	-	1.1
α-Terpinene	1015	1014	-	1.5
Limonene	1029	1020	0.2	-

1,8-Cineole	1033	1032	6.7	15.2
(*Z*)-β-Ocimene	1039	1044	-	1.7
(*E*)-β-Ocimene	1047	1052	0.9	1.7
Terpinolene	1088	1086	-	2.5
cis-*p*-Mentha-2,8-dien-1-ol	1129	1133	-	1.2
Camphor	1146	1141	8.7	10.6
Borneol	1171	1165	15.5	20.0
cis-Chrysanthenyl acetate	1253	1261	21.6	-
Bornyl acetate	1285	1287	2.0	1.2
α-Cubebene	1351	1345	-	0.1
Caryophyllene	1427	1417	-	1.7
trans-α-Bergamotene	1436	1432	-	14.0
(*E*, *E*)-α-Farnesene	1501	1505	-	1.3
β-Sesquiphellandrene	1524	1521	-	0.5
Caryophyllene oxide	1587	1582	-	2.8
Juniper camphor	1683	1690	-	0.7
α-Bisabolol	1687	1685	-	0.7
n-Hexadecanoic acid	1952	1959	5.6	-
Octadecanoic acid	2190	2188	19.5	-
Total			**97.5**	**93.7**
Monoterpene hydrocarbons			**17.9**	**23.5**
Oxygenated monoterpenes			**54.5**	**48.2**
Sesquiterpene hydrocarbons			**-**	**17.5**
Oxygenated sesquiterpenes			**-**	**4.5**
Fatty acids			**25.1**	**-**

[a] Elution order on DB-5 column; RI[b], Retention indices relative to C_9-C_{24} n-alkanes on the DB-5 column; RI [c], Literature retention indices; - Not identified.

4. Conclusions

For the first time, the compositions of the flower and leaf essential oils of the Nigerian grown *C. morifolium* were elucidated. Although, ubiquitous terpenes were identified in all the samples, each species has its own compositional pattern different from others. Factors such as the age of the plant, handling procedure, ecological and climatic conditions etc may have been responsible for the observed compositional differences in the result and other parts of the world.

5. Acknowledgments

Authors are grateful to Mrs. Ogunwande Muslimat for the type setting of the manuscript. The effort of Mr. Daniel Achem of the University of Lagos, Nigeria, in the GC and GC/MS analysis was highly appreciated.

6. References

1. Sun CQ, Huang ZZ, Wang YL, Fa-Di C, Nian-Jun T, Wei-Min F *et al.* Overcoming pre-fertilization barriers in the wide cross between *Chrysanthemum grandiflorum* (Ramat.) Kitamura and *Chrysanthemum nankingense* (Nakai) Tzvel. by using special pollination techniques. Euphytica 2011; 178(2):195-202.
2. Pin-Der D. Antioxidant activity of water extract of four Harng Jyur (*Chrysanthemum morifolium* Ramat) varieties in soybean oil emulsion. Food Chemistry 1999; 66(4):471-476.
3. Guo-Hua L, Lin L, Hua-Wei L, Xin M, Jing-Ye W, Li-Ping W *et al.* Antioxidant action of a *Chrysanthemum morifolium* extract protects rat brain against Ischemia and Reperfusion injury. Journal of Medicinal Food 2010; 13(2):306-311.
4. Wang S, Hao LJ, Zhu JJ, Zhang QW, Wang ZM, Zhang X *et al.* Study on the effects of sulfur fumigation on chemical constituents and antioxidant activity of *Chrysanthemum morifolium* cv. Hang-ju. Phytomedicine 2014; 21(5):773-779.
5. Chong-Kuei L, Yen-Ping L, Hsien-Tsung Y, Yun-Sheng H, Chia-Wen T, Kai-Li L *et al. Chrysanthemum morifolium* Ramat reduces the oxidized LDL-induced expression of intercellular adhesion molecule-1 and E-selectin in human umbilical vein endothelial cells. Journal of Ethnopharmacology 2010; 128(1):213-220.
6. Kim IS, Sushruta K, Pyo-Jam P, Kim EH, Kim CG, Choi WS *et al. Chrysanthemum morifolium* Ramat (CM) extract protects human neuroblastoma SH-SY5Y cells against MPP^+-induced cytotoxicity. Journal of Ethnopharmacology 2009; 126(3):447-454.
7. Lin L, Xin M, Li-Ping W, Lei S, Jie-Qin G, Jun S *et al.* Protective effects of total flavones extracted from chrysanthemum morifolium on rat brain against cerebral ischemia/reperfusion injury. The Journal of the Federation of American Societies for Experimental Biology 2006; 20:A779.
8. Yan C, Xiaoli W, Jie X, Jiangyun L, Meilin X. *Chrysanthemum morifolium* extract attenuates high-fat milk-induced fatty liver through peroxisome proliferator-activated receptor α–mediated mechanism in mice. Nutrition Research 2014; 34(3):268-275.
9. Nguyen TL, Le HT, Tran THH, Pham TB, Nguyen HD, Chau VM *et al.* Inhibitors of α-glucosidase, α-amylase and lipase from *Chrysanthemum morifolium*. Phytochemistry Letters 2013; 6(3):322-325.
10. Fengjie L, Changfeng H, Zhengchun H, Yuanjiang P. An arabinogalactan from flowers of *Chrysanthemum morifolium*: structural and bioactivity studies.

Carbohydrate Research 2014; 387:37-41.
11. Long-Ze L, James MH. Identification of the phenolic components of chrysanthemum flower (*Chrysanthemum morifolium* Ramat). Food Chemistry 2010; 120(1):319-326.
12. Clifford WB, Christopher HJ. Allelopathic activity of luteolin 7-*O*-β-glucuronide isolated from *Chrysanthemum morifolium* L. Biochemical Systematics and Ecology 2005; 33(2):103-111.
13. Shahat AA, Apers S, Pieters L, Vlietinck AJ. Isolation and complete NMR assignment of the numbing principle from *Chrysanthemum morifolium*. Fitoterapia 2001; 72(1):89-91.
14. Lihong H, Zhongliang C. Sesquiterpenoid alcohols from *Chrysanthemum morifolium*. Phytochemistry 1997; 44(7):1287-1290.
15. Toshihiko O, Akinori S, Saburo T, Yuji O, Yoshio S. Molecular structure and stereochemistry of chrysandiol, a novel sesquiterpene diol from *Chrysanthemum morifolium*. Tetrahedron Letters 1974; 15(7):569-1572.
16. Tao W, Qiao-Sheng G, Peng-Fei M. Flavonoid accumulation during florescence in three *Chrysanthemum morifolium* Ramat cv. '*Hangju*' genotypes. Biochemical Systematics and Ecology 2014; 55:79-83.
17. Tao W, Zaibiao Z, Qiao-Sheng G, Peng-Fei M. Variation in major flavonoids glycosides and caffeoylquinic acids during florescence of three *Chrysanthemum morifolium* Ramat cv. '*Hangju*' genotypes. Biochemical Systematics and Ecology 2013; 47:74-79.
18. Wang YJ, Yang XW, Guo QS. Studies on chemical constituents in Huangjuhua (flowers of *Chrysanthemum morifolium*. Zhongguo Zhong Yao Za Zhi 2008; 33(5):526-530.
19. Qing-Lei S, Shu H, Jian-Hui Y, Xin-Qiang Z, Yue-Rong L. Flavonoids and volatiles in *Chrysanthemum morifolium* Ramat flower from Tongxiang County in China. African Journal of Biotechnology 2010; 9(25):3817-3821.
20. Yuan-Yuan X, Dan Y, Jing-Yu Y, Li-Hui W, Chun-Fu W. Cytotoxic activity of flavonoids from the flowers of *Chrysanthemum morifolium* on human colon cancer Colon205 cells. Journal of Asian Natural Products Research 2009; 11(9):771-778.
21. Miyawa M, Hisama M. Antimutagenic activity of flavonoids from *Chrysanthemum morifolium.* Bioscience Biotechnology and Biochemistry 2003; 67(0):2091-2099.
22. Kishimoto S, Ohimiya A. Regulation of carotenoid biosynthesis in petals and leaves of chrysanthemum (*Chrysanthemum morifolium*). Physiologia Plantarum 2006; 128(3):436-447.
23. Wang HK, Xia Y, Yang ZY, Natschke SL, Lee KH. Recent advances in the discovery and development of flavonoids and their analogues as antitumor and anti-HIV agents. Advances Experimental Medicine and Biology 1998; 439:191-225.
24. Beninger CW, Abou-Zaid MM, Kistner ALE, Hallett RH, Iqbal MJ, Grodzinski B *et al.* A flavanone and two phenolic acids from *Chrysanthemum morifolium* with phytotoxic and insect growth regulating activity. Journal of Chemical Ecology 2004; 30(3):589-606.
25. Ragasa C, Tiu Y, Floren RJ. Triterpenoids from *Chrysanthemum morifolium.* ACGC Chemical Research Communications Malaysia: UNESCO 2005; 18(1):11-17.
26. Ukiya M, Akihisa T, Yasukawa K, Kasahara Y, Kimura Y, Koike K *et al.* Constituents of *Compositae* plants. 2. Triterpene diols, triols, and their 3-o-fatty acid esters from edible *Chrysanthemum* flower extract and their anti-inflammatory effects. Journal of Agriculture and Food Chemistry 2001; 49(7):3187-97.
27. Ukiya M, Akihisa T, Tokuda H, Suzuki H, Mukainaka T, Ichiishi E *et al.* Constituents of *Compositae* plants. III. Anti-tumor promoting effects and cytotoxic activity against human cancer cell lines of triterpene diols and triols from edible *Chrysanthemum* flowers. Cancer Letter 2002; 177(1):7-12.
28. Kyung-Mi C, Gun-Hee K. Volatile aroma constituents of *Gukhwa* (*Chrysanthemum morifolium* R). Food Science and Biotechnology 2013; 22(3):659-663.
29. Dong L, Wang J, Deng C, Shen X. Gas chromatography-mass spectrometry following pressurized hot water extraction and solid-phase microextraction for quantification of eucalyptol, camphor and borneol in *Chrysanthemum* flowers. Journal of Separation Science 2007; 30:86-89.
30. Wang YJ, Guo QS, Yang XW, Xu WB, Tao HY. Characterization of chemical components of essential oil from flowers of *Chrysanthemum morifolium* produced in Anhui province. Zhongguo Zhong Yao Za Zhi China Journal of Chinese Materia Medica 2008; 33(19):2207-2211.
31. Guo QS, Wang YJ, Yang XW, Xu WB. Characterization of chemical components of essential oil from cupitulum of *Chrysanthemum morifolium* cultivated in Tongxiang city. Zhongguo Zhong Yao Za Zhi China Journal of Chinese Materia Medica 2008; 33(6):624-647.
32. Jiang HD, Li FG, Zeng S. Capillary GC determination of β-elemene, camphor and borneol in *Chrysanthemum morifolium* Ramat. Chinese Journal of Pharmaceutical Analysis 2005; 25(5):508-511.
33. Zhou HM, Xie PS, Wang WH, Ma JQ, Li P. Analysis of the volatile components from the flowers of *Chrysanthemum morifolium* by GC-MS with solid-phase microextraction. China Journal of Chinese Materia Medica 2005; 30(13):985-988.
34. Lawal OA, Ogunwande IA, Salvador AF, Sanni AA, Opoku RA. *Pachira glabra* Pasq. Essential oil: chemical constituents, antimicrobial and insecticidal activities. Journal of Oleo Science 2014; 63(6):629-635.
35. British Pharmacopoeia Specifications. HM Stationary Office, II, 109, 1990.
36. Adams RP. Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry. Allured, Carol Stream, IL, USA, 2007.
37. Joulain D, Koenig WA. The Atlas of Spectral Data of Sesquiterpene Hydrocarbons. E. B. Verlag, Hamburg, Germany, 1998.