American Journal of Essential Oils and Natural Products
Available online at www.essencejournal.com
AJEONP
American Journal of Essential Oils and Natural Products
AkiNik

ISSN: 2321-9114
AJEONP 2021; 8(3): 39-42

Received: 22-11-2020
Accepted: 24-12-2020

**Thuc Dinh Ngoc**
Department of Science and Technology Management Hong Duc University, 565 Quang Trung, Thanh Hoa City, Vietnam

**Mai Vu Thi Ha**
Department of Science and Technology Management Hong Duc University, 565 Quang Trung, Thanh Hoa City, Vietnam

**Corresponding Author:**
**Thuc Dinh Ngoc**
Department of Science and Technology Management Hong Duc University, 565 Quang Trung, Thanh Hoa City, Vietnam

# Chemical composition of essential oils from the leaves and stems of *Vernonia amygdalina* Del. collected in Vietnam

**Thuc Dinh Ngoc and Mai Vu Thi Ha**

**Abstract**
The chemical composition of the essential oils from leaves and stems of *Vernonia amygdalina* were obtained by hydrodisitillation and analyzed by gas chromatography-mass spectrometry (GC-MS). In this study, total of 31 and 22 compounds were identified, accounting for 94.92% and 73.74% of the total essential oil contents from *Vernonia amygdalina* leaves and stems, respectively. The main components found in leaves oil include caryophyllene oxide (29.51%), *β*-caryophyllene (19.95%), *α*-humulene (8.50%), humulene epoxide II (5.73%), *β*-selinene (4.25%), limonene (3.43%), *α*-selinene (3.28%) and spathulenol (3.11%). While the major components found in stems oil were caryophyllene oxide (36.28%), humulene epoxide II (10.63%), *β*-caryophyllene (4.37%), spathulenol (2.77%), *E*-caryophyllene (2.72%), *β*-selinene (2.53%), *α*-humulene (2.21%) and *β*-cis-elemene (2.09%). This is the first study on the chemical composition of the leaves and stems essential oils of *Vernonia amygdalina* collected in Thanh Hoa province, Vietnam.

**Keywords:** *Vernonia amygdalina*, essential oil, sesquiterpene, caryophyllene oxide, *β*-caryophyllene

## 1. Introduction
*Vernonia amygdalina* is a small shrub tree, distributed in most continents, especially grown mainly in tropical regions such as Africa, China, Vietnam, Malaysia and in some regions of South America [1-4]. This plant has been known to be one of the oldest known medicinal herbs and contains many valuable medicinal compounds. Scientific studies have shown that substances extracted from *Vernonia amygdalina* have different pharmacological properties, including anthelmintic, anti-infection, antimicrobial, anticancer, and antispasmodic properties [5, 6]. Plant leaves are effective in controlling a number of chronic diseases, including glucose and lipid regulatory properties in animals and humans. The extract of *Vernonia amygdalina* leaves contains a variety of substances including terpenoids, saponins, flavonoids, steroids, tannins, anthraquinone, phenols, alkaloids [7, 8]. Some studies on the chemical composition of *Vernonia amygdalina* leaves oil in the South West region of Nigeria showed a total of 20 compounds, accounting for 83.9% of the total oils. The main components of essential oils were thymol (27.0%), (*E*) -phytol (15.7%), ocymene (12.7%), *β*-selinene (8.1%), *γ*-terpinene (4.4%), *β*-caryophyllene (3.9%) and apiole (3.8%) [9]. Another study on the chemical composition of *Vernonia amygdalina* leaves oil in the East region of Nigeria showed a total of 17 compounds, accounting for 89.07% of the total identified essential oils. In which, 1.8-Cineole contains the largest content (25.11%), followed by *β*-pinene (14.54%), myrtenal (6.52%), trans-pinocarveol (6.24%), *α*-pinene (4.93%) and linalool (4.28%) [1]. Thus, two studies on the chemical composition of essential oils from *Vernonia amygdalina* leaves in different regions of a country showed that the main chemical composition of the constituents in essential oil was different.
In Vietnam, there were several studies on *Vernonia amygdalina* plant, an *in vitro* study of *α*-amylase and *α*-glucosidase inhibition of a new steroid saponin from *Vernonia amygdalina* leaves showed that Vernoamyoside E was isolated from this species was able to inhibit *α*-amylase and *α*-glucosidase [10]. Another study on the antioxidant capacity and inhibition of α-glucosidase enzyme of *Vernonia amygdalina* leaves extract showed that extract of dichloromethan had antioxidant ability and inhibition of *α*-glucosidase with content of $IC_{50}$ DPPH = 51.9 µg/mL and 480 µg/mL, respectively [11]. A recent study on the hypoglycemic effect of *Vernonia amygdalina* leaves in Swiss white mice at a dose of 200 mg/kg showed the

strongest hypoglycemic effect and nearly equivalent to the reduction in the group used for comparison with insulin [12]. According to our knowledge, in Vietnam up to now, there has not been any research on the chemical composition of *Vernonia amygdalina* oils. Therefore, the research on the chemical composition of essential oils from the leaves and stems of *Vernonia amygdalina* collected in Vietnam is our goal in this study.

## 2. Materials and methods

### 2.1 Materials

The leaves and stems of *Vernonia amygdalina* were collected from Tho Xuan district (19°95'49.61"N 105°53'36.10"E), Thanh Hoa province, Vietnam in January 2019 and were identified by Prof. Tran The Bach, Institute of Ecology and Biological Resources, Viet Nam Academy of Science and Technology. A voucher specimen (LV-201) was deposited at the Department of Natural Sciences, Hong Duc University, Thanh Hoa, Vietnam.

### 2.2 Extraction of the volatile essential oil

Plant samples after harvesting were washed and cut into small parts of 2-3cm. Essential oils were obtained from leaves and stems of *Vernonia amygdalina* by hydrodistillation using a Clevenger apparatus for 4 h at normal pressure according to the procedure of the Vietnamese Pharmacopoeia until no more essential oil was coming out [13]. The obtained oils were dried over anhydrous $Na_2SO_4$ and stored in sealed vials at 4ºC for GC/MS analysis. The extraction was performed three times with 1 kg of sample each in order to get average result.

### 2.3 Analysis of the essential oils

The chemical composition of the oils were determined Agilent Gas Chromatograph 7980 linked to a MS 5977C mass spectrometer system equipped with a HP-5MS capillary column (30 m x 0.25 mm id. x 0.25 µm film thickness). The temperature was programmed from 60 °C to 280 °C (10 minute hold) at a rate of 4 °C/min. Helium was used as a carrier gas at a flow rate of 1 ml/min. The split ratio was 1: 20, the injector temperature 280 °C. The MS conditions were as follows: ionization voltage 70 eV; emission current 40 mA; acquisitions scan mass range of 35–350 amu at a sampling rate of 1.0 scan/s. Chemical composition research was conducted at the Chemical Analysis Department - Institute of Natural Products Chemistry - Vietnam Academy of Science and Technology. The constituent components were determined by comparing their mass spectra and their Kovats' index (RI) with the oil bank database (NIST 14 version 2.2 and Wiley09) [14].

## 3. Results & Discussion

The essential oils obtained from the leaves and stems of *Vernonia amygdalina* were bright yellow color, mild aroma, content of 0.35% and 0.25%, respectively. 31 compounds from leaves oil and 22 compounds from stems oil were identified, accounting for 94.92% and 73.74% of the total essential oil content of the leaves and stems of *Vernonia amygdalina* plant (Table 1).

**Table 1:** Chemical constituents of the leaves and stems oils from *Vernonia amygdalina*

| No | Compounds | RI[a] | RI[b] | Leaves (%) | Stems (%) |
|---|---|---|---|---|---|
| 1 | *n*-Octanal | - | 1004 | - | 0.30 |
| 2 | *Z*-Hex-3-enyl acetate | 1006 | - | 0.47 | - |
| 3 | Hexyl acetate | 1012 | - | 0.24 | - |
| 4 | Limonene | 1034 | 1034 | 3.43 | 0.75 |
| 5 | Carvone | 1254 | - | 0.12 | - |
| 6 | $\alpha$-Cubebene | 1361 | 1360 | 2.32 | 0.69 |
| 7 | $\alpha$-Copaene | 1390 | 1389 | 2.76 | 0.85 |
| 8 | $\beta$-Bourbonene | 1401 | 1400 | 0.31 | 0.16 |
| 9 | $\beta$-Cubebene | 1403 | - | 2.20 | - |
| 10 | $\beta$-cis-Elemene | 1404 | 1404 | 2.90 | 2.09 |
| 11 | *β*-Caryophyllene | 1438 | 1437 | 19.95 | 4.37 |
| 12 | Calarene | 1446 | - | 0.16 | - |
| 13 | Aromadendrene | 1457 | - | 0.14 | - |
| 14 | $\alpha$-Humulene | 1472 | 1472 | 8.50 | 2.21 |
| 15 | *β*-Chamigrene | 1491 | - | 0.34 | - |
| 16 | $\beta$-Selinene | 1505 | 1505 | 4.25 | 2.53 |
| 17 | $\alpha$-Selinene | 1514 | 1513 | 3.28 | 1.07 |
| 18 | $\beta$-Bisabolene | 1518 | - | 0.12 | - |
| 19 | $\gamma$-Cadinene | 1533 | - | 0.27 | - |
| 20 | $\delta$-Cadinene | 1537 | - | 0.28 | - |
| 21 | *cis*-Calamenene | 1539 | 1539 | 0.22 | 0.27 |
| 22 | $\alpha$-Calacorene | - | 1561 | - | 0.34 |
| 23 | Spathulenol | 1600 | 1599 | 3.11 | 2.77 |
| 24 | Caryophyllene oxide | 1607 | 1606 | 29.51 | 36.28 |
| 25 | Humulene epoxide I | 1621 | 1621 | 0.64 | 0.65 |
| 26 | $\beta$-Oplopenone | 1626 | 1626 | 0.18 | - |
| 27 | Humulene Epoxide II | 1633 | 1632 | 5.73 | 10.63 |
| 28 | 1-Epi -Cubenol | 1648 | 1648 | 0.23 | 0.25 |

| | | | | | |
|---|---|---|---|---|---|
| 29 | Humulene epoxide III | 1654 | 1653 | 0.65 | 1.21 |
| 30 | Caryophylla-3(15),7(14)-dien-6-ol | 1659 | - | 0.75 | - |
| 31 | $\alpha$-Cadinol | 1675 | - | 0.21 | - |
| 32 | *Neo*-Intermedeol | 1678 | 1678 | 0.98 | 1.52 |
| 33 | *Trans*-Calamenen-10-ol | - | 1688 | - | 0.31 |
| 34 | *E*-Caryophyllene | 1692 | 1691 | 0.67 | 2.72 |
| 35 | Cyclocolorenone | - | 1773 | - | 1.77 |
| | Total identified (%) | | | 94.92 | 73.74 |
| | Monoterpene hydrocarbon (%) | | | 3.43 | 0.75 |
| | Oxygenated monoterpene (%) | | | 0.12 | 0.00 |
| | Sesquiterpene hydrocarbon (%) | | | 48.90 | 17.21 |
| | Oxygenated sesquiterpene (%) | | | 41.76 | 55.48 |
| | Non-terpene (%) | | | 0.71 | 0.30 |

$RI^a$, $RI^b$: Kovats index of leaves and stem oils *Vernonia amygdalina*;
- : Not identified.

In the leaves oil, the components with a large proportion were caryophyllene oxide (29.51%), *β*-caryophyllene (19.95%), *α*-humulene (8.50%), humulene epoxide II (5.73%), *β*-selinene (4.25%), limonene (3.43%), *α*-selinene (3.28%), spathulenol (3.11%), *β*-cis-elemene (2.90%), *α*-copaene (2.76%), *α*-cubebene (2.32%) and *β*-cubebene (2.20%). The components with a large proportion in bitter oil are caryophyllene oxide (36.28%), humulene epoxide II (10.63%), *β*-caryophyllene (4.37%), spathulenol (2.77%), *E*-caryophyllene (2.72%), *β*-selinene (2.53%), *α*-humulene (2.21%) and *β*-cis-elemene (2.09%).

For leaves oil, the dominant compound group was sesquiterpene hydrocarbon (18 compounds account for 48.90%), followed by oxygen derivative of sesquiterpene (9 compounds account for 41.76%). While in the stem oil, the dominant substance group is the oxygen-containing derivative of sesquiterpene (9 compounds account for 55.48%), followed by sesquiterpene hydrocarbon (11 compounds account for 17.21%). The same compounds in the leaves and stem oils of *Vernonia amygdalina* plants were that the dominant compounds belong to the sesquiterpene group, the difference was that in the leaves oil, the hydrocarbon sesquiterpene compounds dominated, while in the stems oil, the oxygen derivative compounds of sesquiterpene were dominant.

The chemical composition of *Vernonia amygdalina* leaves oil obtained in Vietnam was completely different from the chemical composition of *Vernonia amygdalina* leaves oil which was studied by Oluwadayo O. *et al* in South Western Nigeria, the obtained leaves oils include thymol (27.0%), (*E*) - phytol (15.7%) and ocymene (12.7%), *β*-selinene (8.1%), *γ*-terpinene. (4.4%), *β*-caryophyllene (3.9%) and apiole (3.8%) [9]. Similar comparison with the study of Aswalam, EF *et al*. showed that the concentration of major substances including 1.8-Cineole (25.11%), β-pinene (14.54%), and myrtenal (6.52%), trans-pinocarveol (6.24%), α-pinene (4.93%) and linalool (4.28%) [10]. The difference in the number of compounds and constituents in essential oils may be due to differences in soil, geology and climate of Vietnam with different regions of study subjects.

## 4. Conclusions

In this study, the chemical constituents of the essential oil isolated from leaves and stems of *Vernonia amygdalina* collected in Thanh Hoa province, Vietnam were determined with the content of 0.35% and 0.25%, respectively. To our knowledge, this is the first study on the characterization of the essential oil composition of this plant from Vietnam. The major chemical components in leaves oil were sesquiterpene hydrocarbons (48.90%), while the main chemical constituents in stems oil were oxygenated sesquiterpenes (55.48%). Researches on the application of essential oils could be investigated in the future.

## 5. Acknowledgments

The authors would like to thank The Director of Analytical Chemistry Laboratory, Institute of Natural Products Chemistry, Vietnam Academy of Science and Technology for GC/MS analyses.

## 6. References

1. Asawalam EF, Hassanali A. Constituents of the essential oil of *Vernonia amygdalina* as maize weevil protectants, Trop. Subtrop. Agroecosyst 2006;6:95-102.
2. Asawalam EF, Emosairue SO, Hassanali A. Contribution of different constituents to the toxicity of the essential oil constituents of *Vernonia amygdalina* (Compositae) and *Xylopia aetiopica* Annonaceae) on maize weevil, *Sitophilus zeamais* Motschulsky, African Journal of Biotechnology 2008;7(16):2957-2962.
3. Nittya K Dogra, Suresh Kumar. A review on ethno-medicinal uses and pharmacology of *Vernoniacinerea* Less., Natural Product Research 2015;29(12):1102-1117.
4. Yeap SK, Ho WY, Beh BK, Liang WS, Ky H, Yousr AHN, Alitheen NB. *Vernonia amygdalina*, an ethnoveterinary and ethnomedical used green vegetable with multiple bioactivities, J. Med. Plants Res 2010;4:2787-2812.
5. Masaba SC. The antimalarial activity of *Vernonia amygdalina* Del. (Compositae), Trans. R. Soc. Trop. Med. Hyg 2000;94:694-695.
6. Ahaiwe UE, Ijeh II, Ejike CECC. Methanolic extract of *Vernonia amygdalina* may protect against experimental benign prostatic hyperplasia in an albino Wistar rat model, J. Med. Aromatic Plant Sci 2013;35:115-120.
7. Abioye VF, Akande EA, Aluko BO. Effects of different local debittering methods on some chemical components and antioxidants in bitter leaf (*Vernonia amygdalina*), Int. J. Res. Chem. Environ 2014;4:96-101.
8. Imaga NOA, Bamigbetan DO. In vivo biochemical assessment of aqueous extracts of *Vernonia amygdalina* (Bitter leaf), Int. J. Nutr. Metab 2013;5:22-27.
9. Oluwadayo O Sonibare, Mubo A Sonibare, Enitan O Adesanya. Essential Oil Composition of *Vernonia amygdalina* Del. from Southwestern Nigeria, Journal of Essential Oil Bearing Plants 2009;12(1):55-58.
10. Anh HLT, Vinh LB, Lien LT, Cuong PV, Arai M, Ha TP *et al*. In vitro study on α-amylase inhibitory and α-glucosidase of a new stigmastane-type steroid saponin

from the leaves of *Vernonia amygdalina*, Natural Product Research 2019, 1-7.
11. Nguyen Thi Chi, Pham Viet Trang, Le Xuan Tien, Nguyen Van Thanh. Study on the ability of α-glucosidase enzyme inhibition and oxidation resistance of leaf extract of *Vernonia amygdalina* Del., family of Asteraceae, Journal of Pharmacology 2018;58(7):25-29.
12. Nguyen Thi Hong Hanh, Doan Thanh Hieu, Tran Thi Hong. The hypoglycemic effect of *Vernonia amygdalina* Del extract. on white mice, Journal of Science and Technology, Thai Nguyen University 2020;225(01):144 - 149.
13. Vietnamese Pharmacopoeia, Medical Publishing House, Hanoi 1997.
14. Adams RP. Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry, ed. 4.1. Allured Publishing: Carol Stream, Illinois 2017.