

RESEARCH ARTICLE

Characterization of *Dysphania ambrosioides* (L.) Mosyakin & Clemants Essential Oil from Vietnam

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Abstract

Dysphania ambrosioides (L.) Mosyakin & Clemants is a fragrant herb widely distributed from warm temperate regions to subtropical and tropical regions of the Northern Hemisphere. The essential oil from *D. ambrosioides* is the main ingredient used in traditional medicine due to its anti-malarial, anti-cancer, and anti-helminthic properties, among others. The chemical composition of *D. ambrosioides* essential oil from the aerial part was extracted by hydrodistillation and analysis by gas chromatography-mass spectrometry, resulting the yield of 0.8%. The main components were characterized as 2,3-dehydro-1,4-cineole (55%), α -terpinene (15.2%), *iso*-ascaridole (15.3%), and *p*-cymene (9.8%), respectively.

Keywords: D.ambrosioides, Essential oil, GC-MS, Hydrodistillation

Introduction

Dysphania ambrosioides (synonym *Chenopodium ambrosioides* L.), known as wormseed, is a herbaceous shrub belonging to the family Amaranthaceae. It is native to Central and South America and distributed mainly from warm temperate to subtropical and tropical climates in the Northern Hemisphere. It finds useful culinary and agricultural uses. In folk medicine, *D. ambrosioides* and its essential oils were used to treat gut worm, limit the growth of some fungi, treat parasite infections in non-ruminant livestock, cats and dogs, and humans for many years (Soares et al., 2017). This herb is widely known in popular medicine as anthelmintic, vermifuge, and emmenagogue and used in the treatment of digestive, respiratory, urogenital, vascular, and nervous disorders (Ávila-Blanco et al., 2014). From 1916 to 1921, in Brazil, more than one million people received the essential oil of *D. ambrosioides* for the control of intestinal worms (Barros et al., 2019). Many studies have reported effects of *D. ambrosioides* essential oil in activities against intestinal worms, leishmaniasis, schistosomiasis and pest insects, which may be attributable to ascaridol (Monzote et al., 2014; Lohani et al., 2012; Kau, 2000; Harraz et al., 2015). In addition, it was shown that *D. ambrosioides* essential oil contained carvacrol, α -pinene, *p*-cymene, α -terpinene, α -terpinyl acetate, and limonene (Harraz et al., 2015; Zhu et al., 2012; Jardim et al., 2008; Sá et al., 2016; Owolabi et al., 2009; Onocha et al., 1999). Ávila-Blanco et al. (2014) evaluated the *in vitro* and *in vivo* antiamebic activity of the essential oil of *D. ambrosioides* in an amoebic liver abscess hamster model. The result showed that the oral administration of essential oil (8 mg/kg and 80 mg/kg) to hamster infected with *Entamoeba histolytica* reverted the infection. In the study of Soares et al. (2017), authors assessed the *in vitro* schistosomicidal effects of *D. ambrosioides* essential oil on *Schistosoma mansoni* and results demonstrated the promising schistosomicidal potential of this essential oil, which had the monoterpenes *cis*-piperitone oxide (35.2%), *p*-cymene (14.5%) *iso*ascaridole (14.1%), and α -terpinene (11.6%) as the major constituents. Result in

research of Ketzis et al. (2002) showed that the essential oil did reduce viability of eggs of *Haemonchus contortus* in vitro, although, short-term treatment of individual goats with the oil were not effective in reducing the number of nematode adults or eggs. Monzote et al. (2014) examined antileishmanial activity of essential oil and its main components against experimental cutaneous leishmaniasis in BALB/c mice. Results demonstrated that the *D. ambrosioides* essential oil showed a better efficacy against experimental cutaneous leishmaniasis caused by *L. amazonensis* in comparison with its pure major compounds.

Futhermore, essential oil of *D. ambrosioides* has been studied as a nature insecticide. Pavela et al. (2018) investigated effect of *D. ambrosioides* essential oil on the housefly, *Musca domestica*. And the results showed that this essential was more toxic to adults of *M. domestica* showing a LD₅₀ of 51.7 µg/ adult. Also, the LC₅₀ values of the essential oil from Chinese *D. ambrosioides* and its active compound (Z) ascaridole against maize weevil adults, *Sitophilus zeamais*, were 3.08 and 0.84 mg/L air respectively (Chu et al., 2011). And they possessed nematicidal activity against the root-knot nematodes, *Meloidogyne incognita* (Bai et al., 2011).

In this study, we aimed to compare the chemical compositions of essential oil of *D. ambrosioides* obtained from the mountainous area of northern Vietnam, where the altitude is from 1200-1500 m above sea level with special weather, with some other studies in the world. According to our knowledge, this is the first study in Vietnam with *D. ambrosioides* essential oil in this region.

Materials and Methods

Plant materials

D. ambrosioides was collected in Lung Phin commune, Bac Ha district, Lao Cai Province, Vietnam (22 ° 36 ' 15 " N, 104 ° 20 ' 55 " E). The plant was collected during flowering season in May-June 2019 and was identified by Assoc. Prof. Tran Huy Thai, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology. A voucher specimen was deposited under the code DG-01 at Organic Biochemistry Laboratory, Institute of Natural Products Chemistry, Vietnam Academy of Science and Technology. After harvesting, the materials were cleaned and had damaged or waterlogged parts removed. Then aerial parts of the plant were dried and stored for extraction.

Extraction of the volatile essential oil

200 g of dried *D. ambrosioides* aerial parts were coarsely ground. Afterwards, the powder was placed in the flask of the Clevenger type hydrodistillation system. The material to water ratio was 1:3 (w/w) and the temperature of the heating mantle was set to 110 °C. The hydrodistillation process took place for 3 hours to afford the mixture consisting of volatiles and water. To separate water from essential oils, anhydrous Na₂SO₄ was used. The essential oil was stored in dark glass bottles for compositional analysis.

Gas chromatography–mass spectrometry analysis

An Agilent Technologies HP7890A GC, coupled with a mass spectrum detector (MSD) Agilent Technologies HP5975C and a DB-XLB column (60 m x 0.25 mm, film thickness 0.25 µm, Agilent Technologies), was utilized to perform GC-MS analysis of the essential oils. The temperature of the injector and detector was initiated at 250 °C and 280 °C respectively. The temperature progress of the column began at 40 °C, increased to 140 °C at 20 °C/min, and then to 270 °C at 4 °C/min. Helium was used as carrier gas and the flow rate was set at 1 mL/min. Injection in the distillation apparatus was performed by splitting with the ratio was 100:1. The volume injected was 1 µL of essential oils. GC-MS was performed under following MSD conditions:

ionization voltage of 70 eV, the emission current 40 mA, acquisitions scan mass range 35-450 amu under full scan. A homologous *n*-alkane series was used as a standard for the determination of retention time indices (RI) of each component in the essential oil sample. The relative percentages (%) of individual components were calculated based on the GC peak area (MSD response) without correction.

Results and Discussion

The yield of *D. ambrosioides* essential oil obtained by hydrodistillation was calculated to be 0.8435%. The afforded essential oil exhibited a pungent odor, bitter taste and pale yellow color. The density of the essential oil ranged from 0.965 to 0.990. Current yield performance is higher than the results obtained with Brazilian and Yemenian *D. ambrosioides*, at 0.3 and 0.52% respectively (dry mass basis) (Jardim et al., 2008; Al-badani et al., 2017). However, much higher extraction yield was reported in a previous study using aerial parts of Chinese *D. ambrosioides*, at 2.12% (Zhu et al., 2012). The yield difference could be attributable on the climatic conditions of the ecological source, the region, the fertility, the status of the material used and the extraction process of the essential oil.

Volatile composition of *D. ambrosioides* essential oil were characterized by GC-MS and results are presented in Table 1. The chromatogram, which signals the presence of constituents through the appearance of peaks, was shown in Figure 1. On the chromatogram, a total of 11 peaks corresponding to 11 active ingredients were identified. By measuring the corresponding peak areas, it was revealed that 11 identified components accounted for 99.13% of the total amount of essential oils and unidentified compounds attributed to 1.17% of total volatile content. The main constituents of essential oils were defined as 2,3-dehydro-1,4-cineole (55%), α -terpinene (15.2%), *iso*-ascaridole (15.3%), and *p*-cymene (9.8%). Other compounds all have content of less than 1%. The chemical composition of the essential oil in the present study is completely different from other previous reports. Specifically, in Owolabi et al. (2009) isolated and identified the main compounds in Nigerian *D. ambrosioides* essential oils, showing that α -terpinene (63.1%) and *p*-cymene (26.4%) were two major components together with less abundant compounds including ascaridole (3.9%), 1,8-cineole (1.3%), isorebidole (3.6%), transverbenyl acetate (0.5%) and γ -terpinene (0.5%). Similarly, Gupta et al. (2002) showed that India *D. ambrosioides* oil contained α -terpinene (63.6%), *p*-cymene (19.5%) and ascaridole (6.2%) as the main components. Another study on compositional variability in volatiles from different pretreatment of *D. ambrosioides* cultivated in Cuba reported that the major components were α -terpinene (17.0-20.7%), *p*-cymene (20.2-21.1%) and ascaridole (30.5-47.1%) (Monzote et al., 2011). On the other hand, ascaridole has been identified as the main compound with largest percentage in *D. ambrosioides* essential oil isolated from Yemen and Madagascar materials. Accordingly, Ascaridole (54.2%), *iso*-ascaridole (27.7%), and *p*-cymene (8.1%) are the main compounds in essential oil in Yemen and *p*-cymene (16.2%), ascaridole (41.8%), *iso*-ascaridole (18, 1%) from Madagascar material (Al-badani et al., 2017; Cavalli et al., 2004). While Ascaridole was absent in the Vietnamese essential oil sample; 2,3-dehydro-1,4-cineole, which is the most abundant compound in this study was absent in Yemen, Madagascar, Nigeria and India samples (Table 2) (Figure 2).

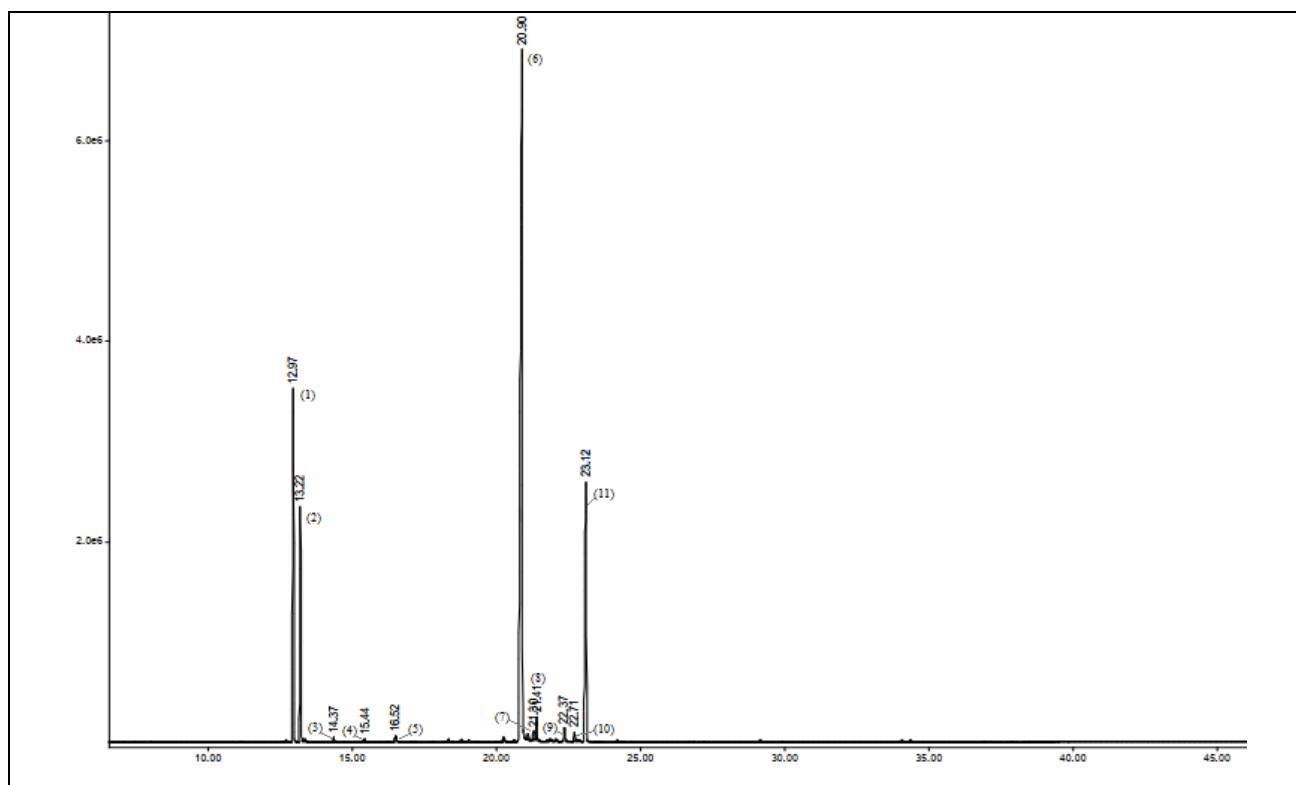
Compositions of essential oils are generally complex and tend to vary with developmental stage of the plant or the climatic conditions in which they are harvested (Zhang et al., 2017). Based on current results and the comparison with the literature, it is suggested that difference in extraction technique may give volatile compositions having contrasting major compounds. In addition, as the ascaridole compound could be easily denatured under elevated temperature (150°C) to form isoascaridole, the discrepancies between reported ascaridole contents could also be attributed measurement errors due to formation of isomers (Cavalli et al., 2004). Another explanation is that interaction between plants and the environment via

secretion and biosynthesis of secondary metabolites may alter essential oil composition and compound quantities (Sá et al., 2016). The diverse biological activity of *D. ambrosioides* essential oils is due to its chemical composition. The highly variable chemical composition of *D. ambrosioides* essential oils with respect to plant part, environment, harvesting time, geographical cultivate, weather, soil, manure sources (Barros et al., 2019; Bibiano et al., 2019).

Table 1. Chemical components of *D. ambrosioides* essential oil

No	RI	Component	%
1	1022	α -Terpinene	15.2
2	1029	<i>p</i> -Cymene	9.8
3	1063	γ -Terpinene	0.2
4	1094	<i>m</i> -Cymene	0.2
5	1125	4-Hydroxy-4-methylcyclohex-2-enone	0.5
6	1251	2,3-Dehydro-1,4-cineole	55.0
7	1263	Piperitone epoxide	0.6
8	1266	unknown (69, 168,)	1.2
9	1294	Menth-2-en-1,4-diol	0.8
10	1304	Carvacrol	0.4
11	1316	<i>iso</i> -Ascaridole	15.3
Total			99.1

Figure 1. Chromatogram of *D. ambrosioides* essential oil.

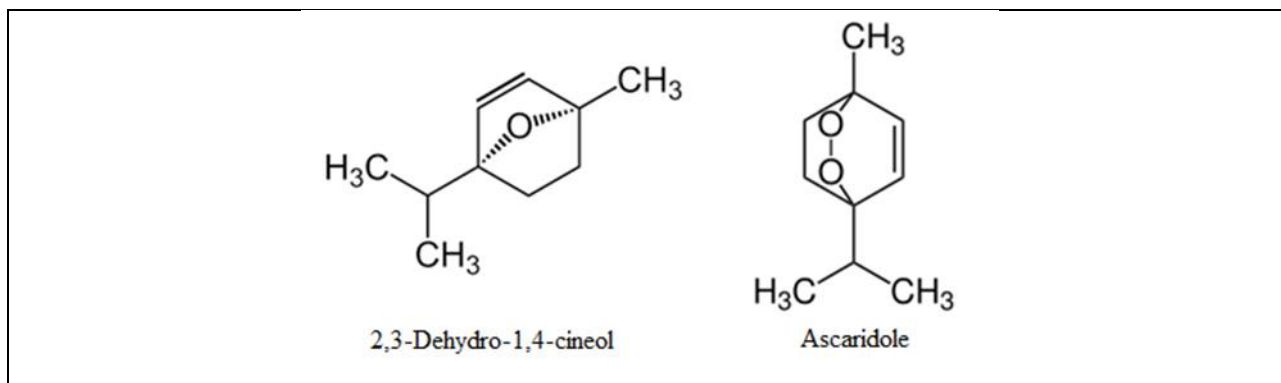


Peak numbers (1-11) are in parentheses: (1): α -terpinene, (2): *p*-cymene,(3): γ -terpinene (4): *m*-cymene, (5): 4-hydroxy-4-methylcyclohex-2-enone, (6): 2,3-dehydro-1,4-cineole, (7): piperitone epoxide, (8): unknown (69, 168, RI 1266), (9): menth-2-en-1,4-diol, (10): carvacrol, (11): *iso*-ascaridole

Table 2. *D. ambrosioides* essential oil composition from different origins

Compounds	Percentage(%)				
	Vietnam (Present study)	Yemen (Al-badani et al., 2017)	Madagascar (Cavalli et al., 2004)	Nigeria (Owolabi et al., 2009)	India (Gupta et al., 2002)
α -Terpinene	15.19	0.70	9.70	63.10	63.60
<i>p</i> -Cymene	9.80	8.10	16.20	26.40	19.50
Ascaridole	-	54.20	41.80	3.90	6.20
2,3-Dehydro-1,4-cineole	54.99	-	-	-	-
iso-Ascaridole	15.31	27.70	18.10	3.60	-

Figure 2: Structures of 2,3-Dehydro-1,4-cineole and Ascaridole



Conclusion

In this study, the chemical composition of the essential oil isolated from aerial parts of *Dysphania ambrosioides* collected in the Lao Cai Province, Vietnam, was determined. To the best of our knowledge, this is the first study on the characterization of the essential oil composition of this species from Vietnam. Essential oil was isolated by hydro-distillation method. The yield of essential oil obtained was 0.8 %. GC-MS analysis was used to identify the chemical compounds of obtained essential oil.

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