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Physico-chemical properties of *Ocimum americanum* L. essential oil from Burkina Faso

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ABSTRACT

The present study aims to determine physico-chemical and organoleptic properties of *Ocimum americanum* L. Essential oil (EO) extracted in Burkina Faso. The protocol described in ISO international standards and Gas Chromatography Mass Spectrometry (GC/MS) were used respectively to determine physico-chemical properties and chemical analysis of the EO. The physico-chemical parameters measured were the acid index, the miscibility with ethanol, the pH, the relative density, the refractive index and the rotatory power. The organoleptic properties studied on the essential oil were the odour, the colour and the appearance. The EO showed a relative density value less than 1, a low light refraction and found to be dextro-rotatory. The pH value obtained was tended towards acidity, the EO was soluble in 5 volumes of ethanol (70%) with a low content of free acids. *O. americanum* EO was fluid, colourless with a specific odour. The chemical analysis of the EO showed that the 1.8-cineole (51.8%) and the camphor (17.4%) were found to be the major constituents of the EO. These results could lead for the establishment of essential oil standards in Burkina Faso.

Keywords: Camphor, GC-MS, ISO international standards, 1.8 cineole.

INTRODUCTION

Approximately 80% of the world’s population use products from plants in official and traditional medicine, where plant products make roughly one-quarter of the total pharmaceutical products (Bhattaram et al., 2002; Tugume et al., 2019). In addition, plant bioactive compounds are widely applied in pharmaceutics industry, cosmetics, food industry, and recently as fine (agro) chemicals and nutraceuticals (Kurek et al., 2022). Among plant extracts, essential oils have a prominent place and are widely used today for their anti-infective, analgesic, anti-inflammatory, sedative, antimicrobial, antispasmodic and antitoxidant, insecticidal, antifungal and acaricidal properties (Morel et al., 2010; Touré, 2015). Hence, they are researched for their applications in food, fragrances, cosmetics, aromatherapy, laundry, home care, pharmaceuticals and others. Essential oils market is growing significantly, exceeding

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USD 7.51 billion in 2018 and is expected to achieve an annual growth rate of 9% between 2019 and 2026 (Sharmeen et al., 2019). Africa has a huge potential for the production of essential oil from aromatic plants. This product is more widely known in the northern part of the continent, but unfortunately Africa's share of the global essential oil market remains weak (FranceAgriMer, 2020). It is therefore important to create a favorable environment for the production and marketing of essential oils in order to take advantage of this vast market. *O. americanum* is an aromatic plant abundantly found in Burkina Faso and represent important medicinal virtues. Various uses and biological activities have been attributed to its essential oil (Kiendrebeogo et al., 2011; Wangrawa et al., 201; Bayala, 2014; Coulibaly et al., 2019). In addition, the plant is used in traditional medicine to treat skin parasitosis, fever and colic (Nacoulma, 1996). In relation to the physico-chemical parameters of *O. americanum* EO in Burkina Faso, there is not much informations in the literature, however these informations are necessary for an efficient use and especially for the marketing of this oil.

It's in that sense the present study aimed at determining physico-chemical properties of O. americanum EO collected in the central part of Burkina Faso.

MATERIALS AND METHODS

Plant material

The plant material consisted of aerial parts (stems, leaves and flowers) of *O. americanum* harvested in September 2021 in the Central region of Burkina Faso (12°25'17" North and 1°29'14" West). A specimen of the plant was authenticated and deposited in the herbarium of the Joseph KI-ZERBO University under the number 6950.

Essential oil extraction

The essential oil was obtained by steam distillation during 3 hours using a Clevenger type apparatus. It was extracted from the fresh plant material. The EO obtained was then dried over anhydrous sodium sulphate and stored in amber bottles at 4°C away from light until using. The following formula was used to calculate the yield value (R) of essential oil:

$$\mathrm{R}\ (\%) = \frac{\text{Essential oil mass (g)}}{\text{Mass of plant material (g)}}\ x\ 100$$

Evaluation of the physico-chemical parameters of the essential oil

Physico-chemical parameters of EO were determined according to the protocol described in ISO international standards 279, 280, 592, 875 and 1242 respectively for the relative density, refraction index, rotatory power, miscibility with ethanol and acid index. The pH of essential oils was determined at 22°C using a pH probe meter (HANNA HI99161). Each parameter was evaluated 3 times.

Relative density (d)

The relative density is the ratio of the mass of a certain volume of the EO to the mass of the same volume of distilled water taken at the same temperature. Density measurement was performed using an analytical balance (Mettler AC 100, Mettler Toledo, Columbus, OH, USA) and a pycnometer of 50 mL. It was calculated using the following formula :

$$\mathrm{d} = \frac{(m2 - mo)}{(m1 - mo)}$$

m_0 = mass of the empty pycnometer in g;

m_1 = mass of the pycnometer filled with distilled water in g;

m_2 = mass of the pycnometer filled with essential oil in g.

Refractive Index (RI)

The refractive index of an EO is the ratio of the sinus of the angle of incidence to the sinus of the angle of refraction of a light ray of a given wavelength passing from the air into the essential oil kept at a constant temperature. It was measured using a refractometer (model ABBE, BOECO, Hamburg, Germany). The refractometer was previously calibrated at 1.333 (at 22°C ± 0.2) corresponding to the refractive index of the standard solution (distilled water). Then the refractive index was measured by placing a few drops of essential oil in the refractometer.

Rotatory power ($[\alpha]_t^\lambda$)

The rotatory power is the measured value of the rotation angle of the polarization plane of light under given experimental conditions. An automatic polarimeter (Mrc-P810, MRC, Holon, Israel) was used to measure the optical rotation of the EO.

Miscibility with ethanol

The ethanol miscibility consisted of determining the volume and the concentration of hydro-alcoholic solution necessary to form a homogeneous mixture with 1 mL of essential oil.

Acid Index (AI)

The acid index is the number of milligrams of potassium hydroxide required to neutralize free acids in 1 gram (g) of essential oil. For this purpose, 2 g of the EO and a maximum of 5 drops of phenolphthalein were added to 5 mL of neutralized ethanol in a saponification flask. After homogenization, the mixture was titrated using a burette with potassium hydroxide solution (0.1mol / L). The acid index was determined according to the formula:

$$AI = V.C \frac{56{,}11}{m}$$

V is the volume, in mL, of potassium hydroxide solution used for the titration;

C is the exact concentration, in mol/L, of the potassium hydroxide solution;

m is the mass in g of the test sample.

pH value determination

The pH was determined by inserting the electrode directly into the sample (essential oil) at an ambient temperature of 22◦C.

Organoleptic properties

The colour and appearance of the essential oil were determined by direct observation and by comparison with other essential oils previously extracted and stored under better conditions. For the odour, the olfactory test was also conducted by comparison with a typical sample of EO from *O. americanum*.

Chemical composition of essential oil

Gas Chromatography–Mass Spectrometry (GC/MS) was used for chemical analysis of the EO. Analyses were performed with a Varian CP-3800 gas chromatography equipped with a DB-5 capillary column (30 m 0.25 mm; coating thickness 0.25 mm) and a Varian Saturn 2000 ion trap mass detector. The analytical conditions were on follows :

- Injector and transfer line temperatures 220 and 240°C, respectively;

oven temperature programmed from 60°C to 240°C at 3°C/min;

- Carrier gas helium at 1 mL /min;
- Injection of 0.2 mL (10% hexane solution);
- Split ratio 1 :30.

Compounds were identified by:

(i) comparison of their retention index (RI) against C5-C24 n-alkanes obtained on a non-polar DB-5MS column, with those provided in the literature and by

(ii) comparison of their mass spectra with those recorded in NIST (National Institute of Standards and Technology) or reported in published papers or by co-injection of available reference compounds.

RESULTS

Physico-chemical and organoleptic properties

The results of Physico-chemical and organoleptic properties of the EO are shown in Table 1. According to chemical properties, the value of acid index was 0.42 mg of KOH / g of EO and the the EO was soluble in 5 volumes of ethanol (70%). For physical properties, The pH value was 3.39, the relative density was 0.90, the refractive index found was 1.345 and the rotatory power was + 27. Finally for organoleptic properties, *O. americanum* EO was fluid and colourless, with a specific odour of thymol.

Chemical composition of essential oil

The results on the chemical composition of *O. americanum* EO analysis are shown in Table 2. A number of 39 chemical compounds were identified of which monoterpenes represented 97.7%. Major components were 1.8-cineole (51.8%) and camphor (17.4%).

Table 1: Physico-chemical parameters organoleptic properties of *O. americanum* EO

	Chemical properties			**Physical properties**				**Organoleptic properties**		
Essential Oil	**AI (mg of KOH / g of EO)**	**Miscibility with ethanol at 22°C**		**pH at 22°C**	**Relative density (d) at 22°C**	**Refractive Index at 22°C**	**Rotatory power (°) at 22°C**	**Odour**	**Colour**	**Appearance**
		Hydro-ethanol mixture (% v/v)	**Number of Volume of hydro-ethanol solution for complete miscibility of EO**							
O. americanum	0.42 ± 0.01	70	5	3.39 ± 0.31	0.90 ± 0.04	1.345 ± 0.21	+ 27 ± 3.21	Specific to camphor	Colourless	Fluid

AI = Acid Index; **EO** = Essential Oil; **KOH** = Potassium hydroxide

Table 2: Chemical composition of *O. americanum* EO.

Terpene groups	Retention Index	Chemical constituents	Pourcentage of components (%)
Monoterpene hydrocarbons	906	Tricyclene	0.1
	911	α-thujene	0.2
	917	α-pinene	1.6
	934	Camphene	0.6
	958	β-caryophyllene	1.9
	963	Myrcene	1.4
	1065	Sabinene	2.3
	1069	β-pinene	0.4
	1088	δ-2-carene	0.2
	1096	P-cymene	1.3
	1101	Limonene	4.7
	1121	Terpinolene	0.2
Oxygenated monoterpenes	1156	Linalool	0.7
	1189	**1.8-cineole**	**51.8**
	1247	**Camphor**	**17.4**
	1267	Terpinene-4-ol	0.6
	1274	Sabinene cis-hydrate	0.3
	1223	Octene-3-yl acetate	0.6
	1249	δ-terpineol	0.4
	1261	α-terpineol	1.7
	1349	Piperitone	0.6
	1378	Bornyl acetate	1.0
	1391	Isopropyl acetate	0.1
	1399	Myrtenyl acetate	0.6
Sesquiterpene Hydrocarbons	1404	α-copaene	0.2
	1421	β-elemene	0.2
	1436	β-caryophyllene	2.4
	1467	α-Trans-bergamotene	1.6
	1488	*(Z)*- β-farnesene	0.4
	1489	α-humulene	0.2
	1513	Germacrene-D	0.6
	1536	β-trans-bergamotene	0.2
	1583	*(Z,E)*- α-farnesene	0.3
	1595	α-muurolene	0.1
	1598	δ-cadinene	0.3

	1609	Elemol	0.3
	1624	*(E)*-nerolidol	0.1
Oxygenated sesquiterpenes	1638	Caryophyllene oxide	0.3
	1641	α-eudesmol	0.1
Total			**98**

DISCUSSION

Extraction yield of the essential oil

The essential oil yield obtained was 0.78%. Other studies showed different results in Burkina Faso (Djibo et al., 2004), Benin (Yayi-ladekan et al., 2011), Brazil (Silva et al., 2015) and Cameroun (Ntonga et al., 2012). These variations in essential oil content could be related to several factors such as geographical situation, plant age, soil type and growing conditions, extraction method, maturation period and effect of drying (Dabire et al., 2011; Tine et al., 2020).

Physico-chemical and organoleptic properties

The physico-chemical and organoleptic properties are important because they allow the characterization and identification of an essential oil. They make it possible to decide their future utilisation.

The relative density value obtained was 0.90 ± 0.04. This result is in agreement with the one of a study conducted in India, where a density of 0.91% was found for this EO (Wouatsa et al., 2014). These values are lower than 1, the density of water. This is a good quality criterion of this EO. Indeed, the majority of essential oils are less dense than water, with the exception of some ones such as clove and cinnamon EOs (Oladimeji et al., 2004). Refractive index is a physical property frequently used to test the purity of oils. The lower the refractive index, the better the quality of the essential oil. The refractive index value was 1.345 ± 0.21. This value was low as it was slightly higher than that of water (1.333). So, the essential oil studied could be considered to be of good quality in terms of purity. A low refractive index of an essential oil indicates its low refraction of light which could favour its use in cosmetic products (Kanko et al., 2004; Tanuja et al., 2021). The rotatory power is an important parameter that is decisive in the control of the naturality of essential oils and for the detection of counterfeits. The enantiomeric composition of the molecules of an essential oil constitutes its identity card, it allows to specify its origin and its biological activity (Aribi-Zouioueche and Couic-Marinier, 2021). The results showed that *O. americanum* EO was dextro-rotatory with a rotatory power value of + 27 ± 3.21. Enantiomeric molecules found in this EO (α-thujene, α-pinene, myrcene, sabinene, β-pinene and p-cymene) were probably the ones that could be used to verify its authenticity. The acid index gives an idea about the free acid content of essential oils. The acid value obtained in this study was 0.42 ± 0.01. This parameter is an important index of physico-chemical properties which is indicates of the age, quality, edibility and suitability of essential oils (Siddiqui et al., 2013). A high acid value indicated that the essential oil is becoming rancid due to storage of the plant under improper conditions or present an adulteration. The acid value obtained in the present study was low (lower than 1). That confirmed that the essential oil was newly extracted and preserved in good conditions. The pH value obtained in our study was 3.39 ± 0.31. This value is in the range of acidity of the pH. The pH plays a key role in chemical and biochemical reactions and can influence the stabilizing properties of an essential oil.

In relation to organoleptic properties, the EO was colourless with a characteristic odour of thymol. According to several authors, the distillation of plant material usually yields a transparent, colorless, or pale-yellow liquid, immiscible with water and having density lower than that of water (Dhifi et al., 2017; Moghaddam and Mehdizadeh., 2017).

Chemical composition of the essential oil

The results of chemical analysis of *O. americanum* EOs obtained were in agreement with those of Djibo et al. (2004) who found the same constituants. However, previous studies shown several chemotypes in this essential oil such as p-cymene (14.61-22.8%) and carvacrol (7.94-30.80%) in Benin (Yayi-ladekan et al., 2011), methylchavicol (46-63%) and linalool (24-33%) in Brazil (Nascimento et al., 2011), linalool (53.8%) and limonene (22.2%) in Cameroon (Ntonga et al., 2012). These different variations in the chemical composition of this EO could be explained by various factors including seasonality, part of the plant used, collection period, temperature, humidity, the sunshine, nature of the soil, nutrient availability and altitude (Soro et al., 2015). These can interfere with a plant’s secondary metabolism and considerably modify the composition of EO. Also, previous studies have revealed that the compounds mainly found in the essential oil of O. americanum (1.8-cineole and camphor) could have numerous biological properties (Marchese et al., 2016; Kowalczyk et al., 2020). This could explain the use of the plant in traditional medicine.

Conclusion

The present study has permitted the determination of physico-chemical properties and chemical composition of O. americanum EO. Physico-chemical properties determined allowed a positive assessment of the essential oil. The chemical analysis revealed mainly the presence of monoterpene compounds in particular 1.8 cineole and camphor. These compounds could explain biological activities of the EO and could have various applications in the food, cosmetic and therapeutic domains. In the future, more advanced analytical methods will be considered (chromatographic, spectroscopic...) to analyze the essential oil and these results could contribute to the establishment of essential oil standards in Burkina Faso.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS’ CONTRIBUTIONS

AC, DMH and MT were determined physico-chemical properties of the EO. IS determined chemical composition of the EO. MK and RCHN supervised the work.

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