PHYSICO-CHEMICAL PARAMETERS AND CHEMICAL COMPOSITION OF ESSENTIAL OIL EXTRACTED FROM *TETRAPLEURA TETRAPTERA* (SCHUM. & THONN.) TAUB. FRUIT PULP

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Abstract
The essential oil composition of Tetrapleura tetraptera ((Schum. & Thonn.) Taub., 1891) dried fruit pulp was analyzed by GC/MS and some physico-chemical parameters such as pH, refractive index, acid index and relative density were determined according to AFNOR methods. Forty-three compounds representing 99.98 % of the oil were identified. The essential oil was dominated by thymol (32.71 %), terpineol (7.54 %), benzyl benzoate (5.87 %), asarone (4.57 %), elemicine (4.11 %), β-selinene (3.36 %) and elemol (3.08 %). Qualitatively, this oil is rich in monoterpenes which represent 47.43 % of the total oil followed by non-terpenic phenylpropanoid compounds (18.90 %). The main monoterpenes are oxygenated aromatic compounds (thymol, terpineol and linalool) with more than 40 % of the total essential oil in the pulp. In addition, sesquiterpenes which are the most numerous represent only 15.76 % of the total oil are oxygenated compounds including spathulenol (0.20 %), δ-eudesmol (0.80 %), α-cadinol (2.49 %) and hydrocarbons including α-copaene (0.25 %), β-elemene (0.21 %), γ-cadinene (0.19 %). Furthermore, the refractive (1.49) and acid (0.99 mg KOH/g oil) indices, relative density at 20°C (0.88) and pH (5.72) obtained were in accordance with the standards published by AFNOR, suggesting an oil of good quality.

Key words: Tetrapleura tetraptera, essential oil, chemical composition, physico-chemical parameters.

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INTRODUCTION

Tetrapleura tetraptera ((Schum. & Thonn.) Taub., 1891) is a deciduous tree of Fabaceae family and belonging to Mimosoideae subfamily. It is generally distributed in the lowland forests of tropical Africa, particularly in western, central and eastern Africa (Ogbunugafor et al., 2017). The tree, which can reach 35 m in height and 2 m in circumference has a smooth and thin bark varying from silver-grey to red. The leaves are bipinnate with 12 pairs of leaflets alternating on each penne. The flowers are creamy pink, sometimes turning to orange, and are solitary or paired in the terminal axils. Mature fruits are green and vary from dark brown to black as they dry (Agbotui, 2015). Dried fruits contain oily, aromatic and sweet substances and are characterized by four longitudinal wing-like veins perpendicular to each other, two of which are pulpy and two of which are woody. This plant, known under several names such as Aridan or Oshosho (Nigeria), Epelekese or Prekese (Ghana), Chêrê-Chêrê or Chêboué (Côte d'Ivoire) is widely used in traditional medicine (Adesina et al., 2016; N'zebo et al., 2018). Indeed, the plant is therapeutically useful in management of convulsions, leprosy, inflammation, rheumatoid pain, diabetes mellitus, arthritis, high blood pressure, epilepsy, asthma, etc.

Due to its extensive use in traditional pharmacopoeia, different parts of the plant have been studied for their biological and pharmacological properties (Orwa et al., 2009; Irondi et al., 2013; Famobuwa et al., 2016). However, with specific regard to volatile compounds of *T. tetraptera*, rare studies on samples of essential oils from the leaves and

dried fruit have been carried out in Cameroon and Nigeria (Ngassoum et al., 2001; Aboaba et al., 2009; Udourioh and Etokudoh, 2014). It also appears that the chemical composition of volatile oils in the pulp of the fruit of *T. tetraptera* has not been studied yet. However, this plant is locally appreciated for its aromatic properties and therapeutic virtues. Therefore, the present work concerned a detailed GC and GC/MS examination of the essential oil of *T. tetraptera* dried fruit pulp. The objective of our study was to determine the chemical composition and to evaluate some physico-chemical characteristics of the essential oil of *T. tetraptera* dried fruit pulp.

MATERIALS AND METHODS

Samples collection and preparation

Plant material used in this work consisted of the pulp of *T. tetraptera* mature dried fruit (Figure 1). Fruits were collected randomly from trees at Loviguié (5°48'24.5" N latitude and 4°20'15.8" W longitude) in Agboville department, southeast Côte d'Ivoire, during the period of availability (August to November). After authentication, fruits were sorted and washed, then the fleshy pulp were extracted using a steel knife.

a

b

Figure 1: Mature dried fruits of *T. tetraptera* (a) and pulp extracted from the fruits (b)

Essential oil extraction

Extraction was carried out by hydrodistillation using a Clevenger-type device. Sorted and cleaned, 500 g of *T. tetraptera* fresh pulp was placed on a wire rack and put in a pressure cooker containing 850 mL of water. This was boiled for 2 h and the essential oils were steamed. After condensation and liquefaction, the oil was separated from the water and then freeze-dried. After extraction, the essential oil mass was determined and then kept away from light and air in a refrigerator (4 °C).

Essential oil yield

Essential oil yield (Y) was estimated by the ratio of essential oil mass (m) to fresh plant material mass (M). It was expressed as a percentage and calculated according to the following formula:

$$Y\ (\%) = \frac{m}{M} \times 100 \qquad (1)$$

Essential oil physico-chemical analysis

Relative density at 20°C (AFNOR NF T-75-111)

The relative density of an essential oil is defined as the ratio of the mass of a certain volume of oil at 20°C and the same mass of distilled water at 20°C. This quantity is

dimensionless and noted d_{20}^{20}. The density was measured using an electronic density meter and the correction at 20°C was made by means of the formula:

$$d_{20}^{20} = dt' + 0.00068\,(t' - t) \quad (2)$$

dt': measured density at temperature t'.
t': temperature at the time of measurement
t: reference temperature which is 20°C

Refractive index (AFNOR NF T-75-112)

This measurement was made with a Prisma-CETI convex refractometer. The refractive index $n_D t$ at the reference temperature t is given by the formula:

$$n_D t = n_D t' + 0.00045\,(t' - t) \quad (3)$$

$n_D t'$: measured refractive index at temperature t'.
t': temperature at the time of measurement
t: reference temperature which is 20°C

Acid number determination (AFNOR NF T-60-2000)

This parameter is defined as KOH mg necessary to neutralize the free fatty acids contained in 1 g of essential oil. The measurement of the acid number (A_N) was carried out by titration where the free fatty acids are neutralized by a titrated ethanolic solution of KOH. The procedure consisted in introducing 0.125 g of essential oil into a flask to which 2.5 mL of 96 % ethanol was added. The mixture was stirred and titrated with an ethanolic solution of KOH (0.1 N), in the presence of phenolphthalein until neutralization characterized by the change from yellow (essential oil) to pink. The volume of the ethanolic KOH solution used for the neutralization was read directly from the burette. The acid number (A_N) is expressed by the formula:

$$A_N = \frac{5.611 \times N \times V}{m} \quad (4)$$

N: normality of KOH.
V: volume in mL of the ethanolic KOH solution used for the titration.
m: mass of the essential oil (g).

Chemical composition of the essential oil of *T. tetraptera* pulp

Chemical composition analysis of the essential oil were carried out by gas chromatography (GC) and then by gas chromatography coupled with mass spectrometry detection (GC/MS) for the determination of their composition. Gas chromatography was carried out using a chromatograph (Delsi DI 200) equipped with a flame ionization detector and a DB5 column (25 m x 0.25 mm, df: 0.25 µm) with a flow rate of 60 mL/min. Nitrogen was used as carrier gas at a flow rate of 1 mL/min. The initial temperature of the column was set at 50°C for 5 min and then increased at 30°C/min to 220°C. The injector temperature was set at 220°C and the detector temperature was set at 250°C. The analyses of the oil by gas chromatography coupled with mass spectrometry were carried out using a gas chromatograph (HP model 6890) coupled with a spectrometer (HP model MS 6890) equipped with an HP5 column (30 m x 0.25 mm df : 0.25 µm) initially programmed at 50°C/5 min. The final temperature of 300°C was reached at an increase of 50°C/min and then stabilized for 5 min. The injection was set in split mode with a division ratio of 1/10. The injector and detector temperatures were 250°C and 320°C respectively. Ionization was performed by electron impact at 70 eV. The electron multiplier was set at 2200 V and the ion source temperature was 230°C. Mass spectrum data were acquired in the scanning mode in the m/z range 33-450. The identification of compounds was carried out by calculating retention indices (RI) or Kovats indices (KI) and were compared with those of the mass spectra in the Adams (2017) databases.

RESULTS AND DISCUSSION

Extraction yield and physico-chemical parameters of the oil

Extraction yield and physico-chemical parameters of the extracted oil is recorded in Table I. The obtained extraction yield (0.03 %) is very low compared to those quoted in literature on various food spices. Indeed, Bahl et al. (2014) and Zheljazkov et al. (2014)

reported extraction yields of essential oil from spices such as turmeric (0.05-1.40 %) and coriander (0.11-0.25 %). Similarly, relatively high extraction yields were reported by Sultan (2005) on ginger (0.85-2.0 %), Chaudhry et al. (2012) on cumin (2.80 %) and Kasim et al. (2014) on cinnamon (1.82 %). The extraction method could partly explain the low extraction rate obtained, as abundant foaming was observed during the boiling of the mixture (water + pulp) making it difficult to extract the essence of the fruit pulp in an optimum way. Also, it is useful to remember that extraction rate of essential oils varies according to the organ, state of freshness, harvest period, extraction technique and even storage during which the loss of most volatile compounds can be important (Bruneton, 2009).

The density of an essential oil is a function of the chemical composition and temperature. It can provide insight into the nature of the product as well as attempts of fraud and adulteration (Laurain-Mattar, 2018). The relative density at 20°C of the essence of *T. tetraptera* fruit pulp (0.88 ± 0.01) complies with AFNOR (2000) standard according to which, the densities of acceptable essential oils are between 0.87 and 0.98, i.e. lower than that of water. However, some exceptions exist with essential oils of cinnamon, clove and sassafras which have higher densities (>1).

Refractive index represents a criterion of oil purity and is used to check the quality of the distillation, because slow distillation at too high temperature lowers the refractive index (AFNOR, 2000). This index depends on the chemical composition and increases with the length of the fatty acid chains, their degree of unsaturation and temperature. It varies mainly with the content of monoterpenes and oxygenated derivatives of oil, a high content of monoterpenes gives a high refractive index (Boukhatem et al., 2010). AFNOR's standard (2000) fixed this index between 1.45 and 1.59 for an essential oil. Thus, the result obtained for *T. tetraptera* pulp essential oil (1.49 ± 0.01) is normative according to French standards and would indicate a quality oil usable in the cosmetic industry.

Acid number indicates as well the degree of preservation as the quality of an edible oil. It is a quality criterion indicating the quantity of free fatty acids present in an essential oil and its sensitivity to alterations including oxidation (Ouis, 2015). It increases with the shelf life during which essential oils could be oxidized and degraded quickly (Boukhatem et al., 2010). AFNOR (1998) recommends that the acid index of an essential oil does not generally exceed 6.5. Thus, the obtained acid index (0.99 ± 0.03) would suggest an oil of slow deterioration quality.

Table I: Extraction yield and physico-chemical parameters of the essential oil of the pulp of *T. tetraptera* fruit.

Parameter	Value	Standard
Extraction Yield (%)	0.03 ± 0.00	-
Colour	Light yellow	-
Outdoor appearance	Liquid and fluid	-
Odour	Aromatic	-
Density d_{20}^{20}	0.88 ± 0.01	0.87 – 0.98 **(AFNOR 2000)**
Refractive index $n_D t$	1.49 ± 0.01	1.45 – 1.59 **(AFNOR 2000)**
pH	5.72 ± 0.01	5 – 6.5
Acid number A_N	0.99 ± 0.03	< 6.5 **(AFNOR 1998)**

Chemical compounds of the essential oil

Forty-three (43) constituents have been identified from *T. tetraptera* pulp essence representing 99.98 % of the total chemical composition of the oil (Figure 2) (Table II). Major compounds accounted for 61.24 % of the essential oil. They were mainly thymol (32.71 %), terpineol (7.54 %), benzyl benzoate (5.87 %), asarone (4.57 %), elemicine (4.11 %), β-selinene (3.36 %) and elemol (3.08 %). Minor compounds were linalool (2.67 %), 1-octanol (2.61 %), 1-decanol (2.50 %), α-cadinol (2.49 %), myristicin (2.40 %), 2,5-dimethoxy-p-cymene (1.80 %), α-linoleic acid (1.45 %), ethyl linoleate (1.38 %), carvacrol (1.33 %), caryophyllene oxide (1.30 %), δ-cadinene (1.28 %), phytol (1.22 %), decanal (1.20 %), geraniol (1.15 %), ethyl palmitate (1.12 %), caryophyllene oxide (1.09 %) and 4-carvomenthol (1.06 %). Qualitatively, this oil is rich in monoterpenes which represent 47.43 % of the total oil followed by non-terpenic phenylpropanoid compounds (18.90 %). Sesquiterpenes which are the most numerous represent only 15.76 % of the total oil (Table III). The main monoterpenes are oxygenated aromatic compounds (thymol, terpineol and linalool) with more than 40 % of the total essential oil in the pulp. Sesquiterpenes are oxygenated compounds including spathulenol (0.20 %), δ-eudesmol (0.80 %), α-cadinol (2.49 %) and hydrocarbons including α-copaene (0.25 %), β-elemene (0.21 %), γ-cadinene (0.19 %). Fatty acids have also been identified in *T. tetraptera* essential oil pulp studied, but in small quantities compared to monoterpenes and sesquiterpenes. These fatty acids are mainly dominated by α-linoleic acid (1.45 %), myristic acid (0.69 %) and octanoic acid (0.36 %). Phytol is the only diterpene present in the chromatogram of the oil with a percentage of 1.22 %. From a functional viewpoint, all these different compounds are divided into organic acids, phenols, alcohols, ketones, aldehydes, carboxylic acids, etc., which would contribute greatly to the observed antimicrobial effects of the fruit (Ekwenye and Okorie, 2010; Oguoma et al., 2015; Ogbunugafor et al., 2017). In addition, a comparative study of the obtained results with data found in literature shows some differences in constitution and thus of some chemotypes in the West African region.

Indeed, in Nigeria, Udourioh and Etokudoh (2014) analyzed the essential oil and fatty acid composition of *T. tetraptera* dried fruit using GC/MS and characterized 44 compounds representing 98.50 % of the oil. The oil was dominated by acetic acid (34.59 %), 2-hydroxy-3-butanone (18.25 %), butanoic acid (8.35 %), 2-methylbutanoic acid (7.58 %), 2-methylbutanol (7.45 %), butanol (4.30%), 2-methylbutenoic acid (3.65 %) and nerol (3.25 %). Similarly, the chemotype from Cameroon analyzed by Ngassoum et al. (2001) showed five major compounds in the fresh fruit, namely acetic acid (48.20 %), 2-methyl-2-butenoic acid ethyl ester (10.30 %), 2-hydroxy-3-butanone (8.10 %), 2-methylbutanoic acid (8.10 %) and 2-methylbutanol (6.10 %). These authors also showed that dried fruit oil was dominated by 2-hydroxy-3-butanone (31.60 %), 2-methylbutanoic acid (14.50 %), acetic acid (13.00 %) and butanoic acid (9.50 %). In another report from Nigeria, forty-one (41) compounds representing 89.50 % of the essential oil of *T. tetraptera* leaves were characterized after GC/MS analysis (Aboaba et al., 2009). This essential oil was dominated by 1,8-cineole (19.40 %), 6,10,14-trimethyl-2-pentadecanone (13.60 %), phytol (9.10 %), α-pinene (8.10 %) and geranyacetone (6.70 %).

The chemotype obtained in the present study after analysis of the essence of *T. tetraptera* dried fruit pulp differs strongly from previous reports on the plant with a predominance of thymol (32.71 %). These differences could be due to the method of extraction, the soil and climate conditions, the ripeness (age) of the fruit or the part of the plant analyzed.

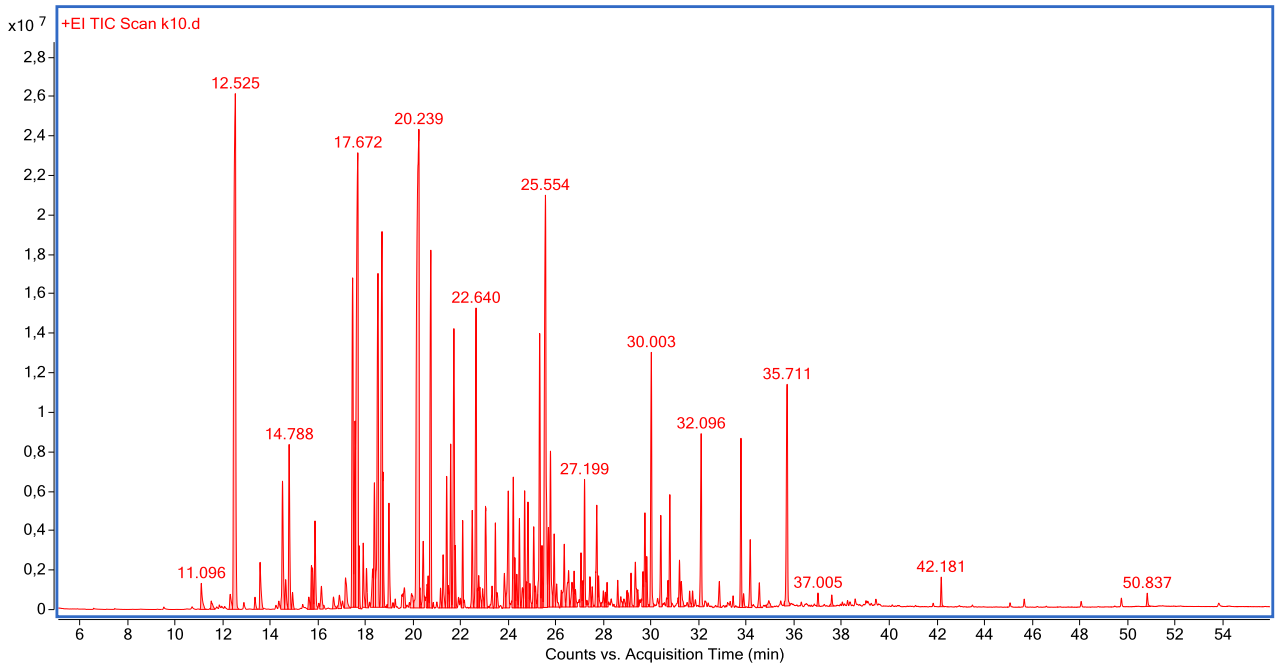

Figure 2: Chromatogram of the essential oil extracted from the pulp of the mature dried fruit of *T. tetraptera*.

Table II: Chemical composition of the essential oil extracted from *T. tetraptera* dried fruit pulp

Peak	Identified compound	Ri[a]	Ri[b]	Concentration (%)
1	**5-ethyl-2-methylthiazole**	**983**	**1122**	**4.60±0.10**
2	d-limonene	1020	1146	0.39±0.00
3	1-octanol	1052	1185	2.61±0.40
4	linalool	1081	1219	2.67±0.40
5	nonanal	1088	1225	0.16±0.00
6	octanoic acid	1154	1290	0.36±0.10
7	umbellulone	1159	1304	0.18±0.00
8	menthol	1159	1311	0.19±0.00
9	4-carvomenthenol	1161	1316	1.06±0.20
10	**terpineol**	**1198**	**1332**	**7.54±0.80**
11	decanal	1209	1337	1.20±0.10
12	geraniol	1232	1388	1.15±0.10
13	1-decanol	1256	1412	2.50±0.30
14	**thymol**	**1266**	**1436**	**32.71±1.10**
15	carvacrol	1272	1443	1.33±0.10
16	piperitenone	1304	1495	0.21±0.00
17	α-copaene	1356	1539	0.25±0.00
18	β-elemene	1382	1552	0.21±0.00
19	methyl eugenol	1389	1554	0.15±0.00
20	dodecanal	1389	1563	0.43±0.10
21	2,5-dimethoxy-p-cymene	1399	1569	1.80±0.30
22	caryophyllene	1424	1592	1.09±0.20
23	α-humulene	1456	1632	0.25±0.00
24	myristicine	1482	1667	2.40±0.00
25	**β-selinene**	**1488**	**1670**	**3.36±0.10**

26	γ-cadinene	1513	1693	0.19±0.10
27	δ-cadinene	1519	1697	1.28±0.10
28	**elemol**	**1536**	**1730**	**3.08±0.10**
29	**elemicin**	**1548**	**1737**	**4.11±0.10**
30	spathulenol	1576	1769	0.20±0.00
31	caryophyllene oxide	1578	1778	1.30±0.00
32	δ-eudesmol	1596	1830	0.80±0.00
33	δ-cadinol	1618	1844	0.77±0.00
34	α-cadinol	1650	1855	2.49±0.10
35	**asarone**	**1678**	**1859**	**4.57±0.10**
36	myristic acid	1748	1949	0.69±0.00
37	**benzyl benzoate**	**1758**	**1979**	**5.87±0.20**
38	phytone	1817	2044	0.49±0.00
39	pentadecanoic acid	1823	2061	0.21±0.00
40	ethyl palmitate	1966	2210	1.12±0.00
41	phytol	2104	2346	1.22±0.00
42	α-linoleic acid	2115	2369	1.45±0.10
43	ethyl linoleate	2139	2399	1.38±0.00

Ri^a: retention index obtained on non-polar column; Ri^b: retention index obtained on polar column

Table III: Different classes of the main compounds identified in the essential oil of *T. tetraptera* fruit pulp

Compound class	Number of compound	Concentration (%)
Monoterpenes	10	47.43
Sesquiterpenes	14	15.76
Diterpenes	1	1.22
Phenylpropanoids	6	18.90
Other oxygen compounds	4	4.29
Fatty acids and fatty acid esters	6	5.21
Others	2	7.21

CONCLUSION

The essential oil of *T. tetraptera* mature dried fruit pulp is rich in alcoholic terpenes with thymol and terpineol in particular, which contribute to the aromatic profile of the fruit. In general, this work contributes to a better knowledge of the chemical compounds contained in the essential oil of *T. tetraptera* whose dried fruit is used locally both in food and in traditional medicine.

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