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CHARACTERISTICS, FATTY ACID PROFILE, STRATEGIC IMPORTANCE OF *ZANTHOXYLUM ZANTHOXYLOIDES* (RUTACEAE) SEED OIL AND SUSTAINABLE CONSERVATION OF THE SPECIES

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ABSTRACT

Zanthoxylum zanthoxyloides Lamb (Rutaceae) is a threatened plant with medicinal and pesticidal properties. Its roots are over-harvested. The objective of the study was to determine the profile and the potential beneficial biological properties of its seed oil. The average weight of the seed was 22.98 ± 0.51 mg. It contained 5.5% water and 38.5% oil. Chemical analysis of the oil by gas chromatography coupled with mass spectrometry revealed the presence of a monounsaturated fatty acid, oleic acid, in the proportion of 56.5% and two polyunsaturated fatty acids. These acids are: linoleic acid or omega-6 and linolenic acid or omega-3 in the same proportion of 4.8% each. *Z. zanthoxyloides* seed oil is of oleic type with an unsaturated fatty acid content of 67.6%. The saturated fatty acid content was 30.8%. It is a high quality oil because of the high proportion of unsaturated fatty acids and the presence of two essential fatty acids which confer beneficial effects on many physiological processes. Study of biological properties of the oil must be enlarged to permit to substitute the exploitation of the root bark with that of the seeds. Use of seeds instead of roots insures best conservation of this medicinal plant species.

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INTRODUCTION

Zanthozylum zanthozyloides Lamb (Rutaceae) is a shrub or tree, depending on its habitat and has broad-based spines on the twigs and rachis of the leaves. As a savanna and dry forest plant in West Africa, *Z. zanthoxyloides* is also abundant in coastal areas; which makes it a littoral species as well In Togo it grows in the mangroves of Agbanakin, on the sandy soils of Noèpe and the clay soils of Agou (Brunel *et al.,* 1984). In the Maritime Region of Togo, flowering and fruiting occur during the rainy season; ripening of the fruits taking place towards the end of the season. The fruits form green bunches that turn red when they are ripe. Harvesting of ripe fruits can spread from October through December, depending on rainfall and age of trees. Older trees bear fruit earlier. *Z. zanthoxyloides* is well known in West Africa for its therapeutic virtues.

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The leaves are used as an antibiotic to treat incurable wounds (Le Grand, 1989). The essential oil obtained by hydro-distillation of dried fruits controls gastrointestinal parasitosis in sheep (Azando *et al.,* 2017). The bark of the root is used to treat toothache, edema, intestinal worms and to stimulate milk secretion in women (Le Grand, 1989). According to Oriowo (1982), the plant has anti-inflammatory and anti-panicle properties. The bark of the stem or root induces local anesthesia of the oral mucosa due to the presence of isobutylamide pellitorine. The pericarp of the fruit contains, per 100 g: 90 mg of Ca, 41 mg of P, 2 mg of Fe, 10 mg of Na, 46 mg of K, 52 mg of Mg and 55 mg of Cu (Matu 2011). In addition to its therapeutic properties, *Z. zanthozyloides* and species of this genus are a potential source of insecticidal products (Kubo 1993). Indeed, several compounds with insecticidal properties have been identified in the fruit pericarp and in the bark of the root. Thus, Adesina (1986) identified pellitorine, fagaramide and other amides with insecticidal properties in these organs. The active compounds can act alone or in synergy. Miyakado *et al.,* (1980) and Nuto (1995) described the synergistic effect of pellitorine and piperine on *Callosobruchus chinensis* and the synergistic effect of pellitorine and fagarasterol on *Callosobruchus maculatus* F. respectively. Pellitorine and fagaramide have repellent properties against termites and the larvae of the lepidoptera *Spodoptera litura* (Escoubas *et al.,* 1994). According to Bawa (2000), the powder delayed mold growth in cowpea stocks in laboratory. These observations reveal the potential importance of *Z. zanthoxyloides* biopesticides in the protection of foodstuff stored in tropical zones. Overall, *Z. zanthoxyloides* is an important source of diverse products used or usable in human health and the protection of agro-resources. This is justified by the great diversity of products and uses contained in the 50 plant references reported in the 'Prelude Data Bank' of the Royal Museum for Central Africa in Belgium (http://www.africamuseum.be, 2018). The range of products from the plant includes: essential oils of leaves and fruits, aqueous, alcoholic or hydro-alcoholic extracts of root bark (Azando *et al.,* 2017; Ynalvez *et al.,* 2012; Zahoui, 2010). According to Negi *et al.,* 2011 citing Kritikar and Basu (1983), the fruits of *Zanthoxylum* species are used as digestive appetizer, to eliminate pain and to cure many diseases like heart diseases, piles, diseases of mouth, teeth and throat. Such observations imply the chemical study of the different parts of the fruit such as the pericarp and the seed. So, the chemical components involved in their biological properties could be identified clearly and the traditional uses scientificaly justified. Chemical compounds of the essential oil of the fruit and its pericarp are well documented. Unfortunately, those of the seed oil are almost unknown. Therefore, the study of this vegetable oil becomes a real necessity. . Zhang and Jiang (2008) studied the seed oil of *Zanthoxylum bungeanum* for biodiesel production purposes. With the exception of the leaves and fruits, the exploitation of the roots represents a danger to the plant because it contributes to the elimination of the species in the long term. *Z. zanthoxyloides* is now considered a threatened plant species in Togo. To prevent such a disaster while benefiting from the biological properties of this well known medicinal plant, the strategy must include the study of the biological properties of its seed oil. The aim is to determine if the oil could reveal properties similar to or more interesting than those shown by the root bark. The root and seed are reserve organs in certain plants. Therefore, an examination of the chemical composition and biological properties of the seed oil becomes imperative. For Smita *et al.,* (2017), primary screening of phytochemicals is a crucial step, in the detection of the bioactive compounds present in medicinal plants. The first step of this study is to examine the chemical composition of the oil, which is the subject of the present work.

MATERIALS AND METHODS

Seed collection sites: The main sites for harvesting ripe fruit are located in the Koudassi sector (02 ° 63'52"N, 07 ° 33'97"E) in Ave district in the Maritime region of Togo. The sites are in ecofloristic zone V.

Characteristics of the seeds: The fruits are dehiscent and release, on drying, tiny pinehead-sized seeds. The seeds were dried in the sun for 3 to 4 days.

Determination of the weight of the seeds: A handful of seeds was left to dry in the sun until their weight stabilized. Five batches of one hundred seeds each were sampled and weighed. The average weight of one seed was determined in each of the 5 batches. The average weight of a seed was calculated from the 5 weights.

Determination of the water content of seeds: It was done according to the DGF B 14 method indicated in Table 1.

Extraction of the oil

Determination of the oil content of seeds: It was done according to the DGF B 15 method indicated in Table 1.

Extraction of oil: Extraction of the oil was done on 5g of seed powder with petroleum ether in a soxhlet device for 5 hours. After extraction, traces of petroleum ether were evaporated in a rotary vacuum evaporator for 3 hours.

Fatty acid composition of the oil: The analysis was done in "Laborservice Laborentwicklung Baugrundanalytik" laboratory in Wittenberge, Germany. The parameters examined and the methods used are those defined by the German Society of Oil Science (Deutsche Gesell-schaft fur Fettwissenschaften). They are indicated in Table 1. Fatty acid composition is determined by gas chromatography coupled with mass spectrometry according to the DGF C VI 10a method (Table 1). The oil was analyzed in the German laboratory indicated above by gas chromatography. The chromatograph was equipped with an ionizing flame detector equipped with a fused silica capillary column (30 m × 0.25 mm covered with a DB23 film 0.5 µm thick); operating temperature was 260 °C; the carrier gas was nitrogen, with a flow rate of 1ml/min. Identification of the oil constituents was done on the basis of comparison of their retention indices and their mass spectrometry (MS) with those of the reference fatty acids. The technical data sheets for the methods used can be obtained from "Laborservice Laborentwicklung Baugrundanalytik " in Wittenberge, Germany.

Table 1. Parameters analyzed and the method used for each analysis. The analysis was done in 2003 in ''Laborservice Laborentwicklung Baugrundanalytik'', Wittenberge, Germany

Parameters	Methods used
Water content of seeds	DGF B 14
Oil content of seeds	DGF B 15
Saturated and unsaturated fatty acids	DGF C VI 10 a

RESULTS

Characteristics of seeds

Seeds appear black with bluish reflection: The apparent richness of the seed in oil is perceptible to the touch. However, it easily succumbs when pressure is applied and crumbles into small fragments to give a simple powder, unlike the peanut seed that forms a paste when crushed. Its flour is milky white with blackish fragments from the disintegration of the integument. Its weight, water and oil contents are shown in Table 2. The oil content of the seed is high, 38.5% against 22% for soybean seed

Characteristics of the oil: *Z. zanthoxyloides* seed oil has a light yellow color. The results of chemical analysis of the oil are shown in Table 3. The analysis was carried out in the "Laborservice Laborentwicklung Baugrundanalytik" laboratory in Wittenberge, Germany.

Table 2. Characteristic parameters of *Z. zanthoxyloides* seed

Parameters	Values
Average weight of seed	22.98 mg ± 0,51
Water content of seed	5.5%
Oil content of seed	22%

Table 3. Fatty acid profile of *Z. zanthoxyloides* seed oil

Major fatty acids	Content (%)
Palmitic acid (C 16 :0)	28,0
Palmitoleic acid (C 16 :1)	1,0
Stearic acid (C 18 :0)	2,3
Oleic acid (C 18 :1) (omega 9)	56,5
Linoleic acid (C 18 :2) (omega 6)	4,8
Alpha-linolenic acid (C 18 :3) (omega 3)	4,8
Saturated fatty acids	30,8
Unsaturated fatty acids	67,6

Table 4. Oil profile of Z. zanthoxyloides seeds. Chromatographic analysis by GC / FID after trans-esterification (LEXVA ANALYTIQUE, France, 19/09/2017)

Major fatty acids	Percentage FID
Palmitic acid (C 16 :0)	23.232
Palmitoleic acid (C 16 :1)	0.633
Stearic acid (C 18 :0)	2.971
Oleic acid (C 18 :1) (omega 9)	50.101
Linoleic acid (C 18 :2) (omega 6)	8.429
Alpha-linolenic acid (C 18 :3) (omega 3)	9.645
Arachidic acid	0.210
Cis-11-eicosenoic acid	0.416
Behenic acid	0.526
Erucic acid	0.218
Tricosanoic acid	1.053

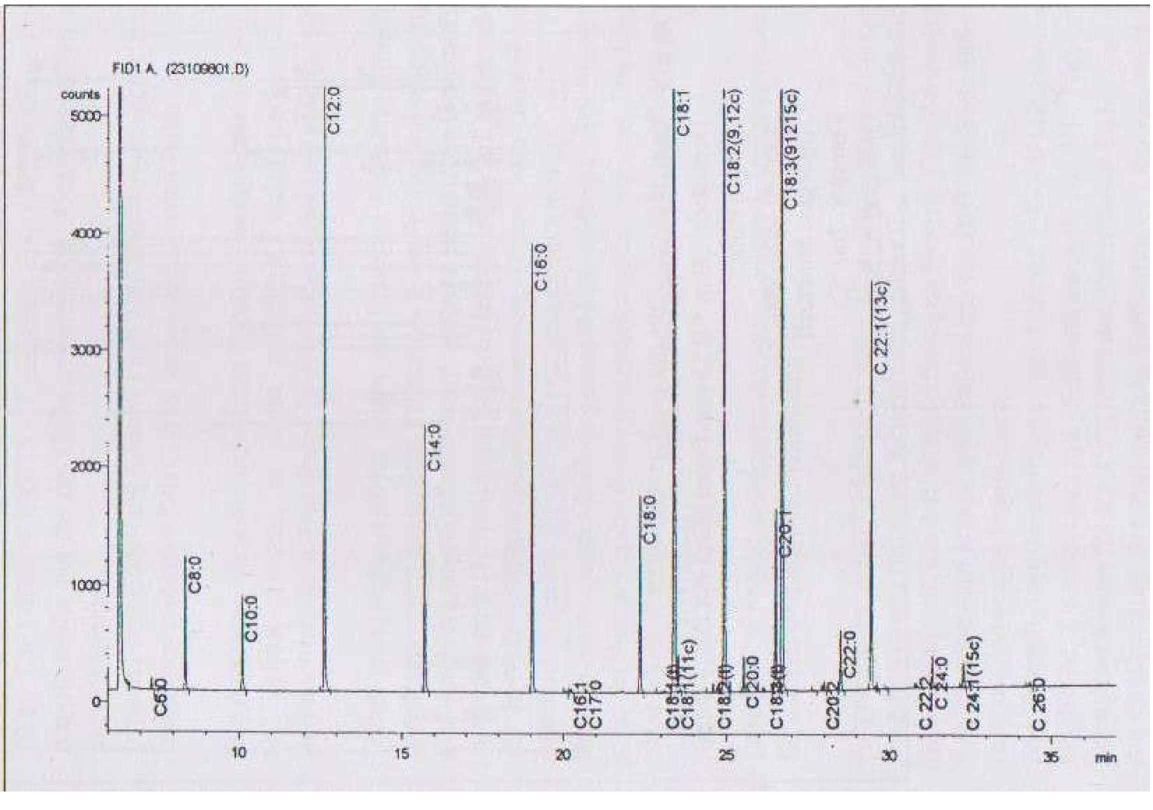

Figure 1. Chromatogram of fattyacids in *Z. zanthoxyloides* seed oil

The chromatogram of the main fattyacids in the oil is shown in Fig. 1. Any other information relating to the chromatographic analysis of the oil can be obtained from this laboratory. The results in Table 3 show that the oil is rich in unsaturated fatty acids. It is an unsaturated oil of oleic type. It can be considered a high quality oil because of its high content (67.6%) of unsaturated fatty acids. It contains two essential fatty acids: linoleic acid: C 18: 2 (9, 12) or omega-6 and linolenic acid: C18: 3 (9, 12, 15) or omega-3. GC / FID chromatographic analysis after trans-esterification of another sample of the oil in 2017 confirms the profile of the oil; that is, an oleic type oil (Table 4).

DISCUSSION

The present study provides insights into the fatty acid composition of the seed oil of *Z. zanthoxyloides,* thereby enriching the repertoire of chemical compositions of the various organs of the species. The seed does not give a pasty mash as kernel nut or peanut seed. *Z. zanthoxyloides* seed content in oil is quite high (38.5%) compared with soybean (22%). The presence of two essential fatty acids: linoleic acid and linolenic acid brings it closer to sesame, palm kernel, cotton, corn, soybean and rapeseed oils that have varying contents of each of the two essential acids (Cuvelier *et al.,* 2004). However, it is more complete than olive oil and coconut oil which contain only linoleic acid (Cuvelier *et al.,* 2004). The high level of polyunsaturated fatty acids makes the oil exploitable for human consumption. But it must be eaten fresh to avoid the appearance of free radicals under the effect of heat. According to Matu, (2011), the seeds of *Z. zanthoxyloides* have a strong cinnamon or peppery taste and are generally used to season food. The polyunsaturated acids in the oil give it anti-inflammatory properties recognized for these acids, as well as their potential to prevent cardiovascular and certain neurodegenerative diseases (Couëdelo *et al.,* 2017).Therefore, use of the oil for its anti-inflammatory properties can be considered. For external uses, one must avoid exposure to sunlight to prevent the appearance of free radicals under the effect of sun rays, especially ultraviolet rays. The high content of poly-unsaturated fatty acids makes the oil or the seeds a very good ingredient in animal feed, especially poultry feed. Indeed, Kouakou *et al.,* (2017) have shown that the incorporation of euphorbia (*Euphorbia heterophylla*) seeds, a source of polyunsaturated fatty acids, including alpha-linolenic acid or omega-3 in the commercial food of laying hens enriches the egg yolk in omega-3 and improves the nutritional quality of quail egg. Kouakou *et al.,* (2012) also showed that the omega-3 content of guinea pig (*Cavia porcellus* L.) meat was significantly improved when the same plant is incorporated into the diet of these animals. According to Dordevic *et al.,* (2016) adding linseed to the diet of pigs induced a variation in the content of omega-6 and omega-3 fatty acids in their meat and backfat with improvement of the omega-6/omega-3 ratio. Adding linseed to the diet of dairy cattle increased the content of their milk fat in some monounsaturated and polyunsaturated fatty acids (Suli *et al.,* 2018). Our results reveal a very interesting fatty acid profile of the vegetable oil of *Z. zanthoxyloides* seed, related to the presence of polyunsaturated fatty acids, in particular omega-3 and omega-6. This increases the biological and therapeutic value of *Z. zanthoxyloides* already known in the sub-region as a medicinal plant (http://www.africamuseum.be, 2018). The valorization of the seed through its vegetable oil and its cake could contribute to satisfy the growing needs for vegetable oils and proteins; these needs being economic and technological stakes for the reinforcement of food security in the world (Anonymous, 2017). The study of the biological properties of the oils hould be extended to its effects on sickle disease just as it was done with the root bark. It could also consider the pathogenic microbes of the bucco-pharyngeal system that are sensitive to extracts of the root bark (Agbulu *et al.,* 2015).Given that many polyunsaturated fatty acids are known to have antibacterial and antifungal properties (Agoramoorthy *et al.,* 2007), an antimicrobial study could reveal properties similar to those of root bark extracts and perhaps, even more interesting properties. It's possible effectiveness on these pathogens would make it possible to offer bucco-pharyngeal cavity care products based on the oil to replace toothpicks made from the roots of the plant. In this context, only the seeds will be exploited; thus avoiding precipitating the elimination of the plant through overexploitation of the roots whose barks are still massively sold in local markets in several West African countries like Ivory Coast, Ghana, Mali, Burkina Faso, Nigeria and Togo. Using the seed oil has many advantages for the oil content of the seed is high, seed collection does not affect the survival of the plant and a large quantity can be collected. The seeds must be collected during an appropriate time period for the fruit is dehiscent.

Conclusion

The present study has proven that *Z. zanthoxyloides* seeds are an excellent source of fatty acids. It is imperative to examine the biological properties of the oil of the seed and to compare them with those of its root bark. Such studies may reveal more interesting biological properties than those recognized for the root bark. This could divert the massive exploitation of the roots to that of the seeds, reducing the exposure of the species to abusive and dangerous exploitation. In addition to the knowledge of the fatty acid composition of the oil, a broad knowledge of its chemical composition and biological properties would enrich and enhance the sustainable use of *Z. zanthoxyloides* for a wide range of purposes.

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