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Oilseeds Potential of *Zanthoxylum zanthoxyloides* (Lam.) Zepernick and Timler

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ABSTRACT

The aim of this prospective study was to assess firstly oil potential of Zanthoxylum zanthoxyloides fruit and then the fatty acid profile. After extracting the oil from the seeds of this plant by the soxhlet method using petroleum ether as solvent, we proceeded to analyze the profile of fatty acids by gas chromatography with derivatization. A very interesting oil yield of 41.8 ± 4.0% was measured and highlighted a total of thirteen fatty acids with six saturated fatty acids and seven unsaturated fatty acids at varying proportions. This oil contained a high proportion of unsaturated fatty acids (72.4%) and the major unsaturated fatty acids are oleic acid (46.4 ± 12.7%)), linoleic acid (12.8 ± 3.5 %) and alpha-linolenic acid (11.7 ± 3.2%). This oil has a particular fatty acid profile due to the presence of three essential fatty acids namely linoleic acid (ω6) and alpha-linolenic acid (ω3) in large quantities and very small quantities of gamma linolenic acid (ω6).

Key words: *Zanthoxylum zanthoxyloïdes, oilseed, fatty acids.*

INTRODUCTION

In sub-Saharan Africa many plant oils are nowadays used in industry [6,9]. In Burkina Faso in 2014, production of groundnut, sesame, soy and cotton seed were respectively 335,223; 321,837; 15,055 and 894,982 metric tons[7]. These speculations are the main sources of edible extracting oil in the country. Despite this production, Burkina Faso remains an oil importing country[12], hence the need to diversify sources of production. Many species found in African flora are still underutilized, even unrecognized by populations[13]. Yet these species are potential sources of new edible oils. This is the case of *Zanthoxylum zanthoxyloides* (Lam.) Zepernick and Timler. No study made on the seeds of this species has mentioned it as the oleaginous species. *Zanthoxylum zanthoxyloides* belongs to the family of Rutaceae. It is a shrub or small tree, prickly reaching 12 meters high.

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The bark is gray to beige, rough, with thin vertical cracks and often woody protrusions wearing spurs. The leaves are alternate, smooth, imparipinnately compound, 5 to 11 leaflets opposite or alternate and with many glandular points. The inflorescence is a terminal or axillary panicle lose 5-25 cm long, ovoid fruits are dehiscent follicles, 5-6 mm in diameter, red and shiny black seeds[3]. This plant is found in the tropical zone of West and Central Africa [2]. In Burkina Faso, this species is very common in some Southwest flora[14].

The goal of this work is to highlight the oleaginous potential of the seeds of this plant and to determine the fatty acid profile of its oil. This work is in relationship with the characterization and the valuation of the Burkina Faso plant resources.

MATERIALS AND METHODS

Plant material

Zanthoxylum zanthoxyloides fruits were harvested in the natural forests of Dindérosso and Kua, two places located around the city of Bobo-Dioulasso. In these places, flowering of *Zanthoxylum zanthoxyloides* begins in July and then fruits (Figure 1) are available from October until March.

Fig.1: Ripe fruits of *Zanthoxylum zanthoxyloides* (Source: Ouoba, 2006)

Seed harvesting

The fruits were harvested during November and December. In the laboratory the fruits were dried and stored away from light. When the follicles are dry they open to release the seeds.

Seed oil extraction

Dried seeds of *Zanthoxylum zanthoxyloides* were milled with a crusher type Retsch GM-200. Homogenates obtained were used for extraction of the fat matter. The extraction was done using a soxhlet extractor according to the NF EN ISO 659[10]. For this, the cartridges (HM1004-33x94mm) were filled with 200g grams of the homogenate and placed in the extractor column. As extracting solvent, petroleum ether was used (Carlo Erba SDS-REACTIVE), an apolar solvent. After 4 hours,

the extract (mixture of ether and fat) was evaporated (Rotavapor R-200 BÜCHI) to remove the solvent. The experiment was repeated three times. The fat, stripped of solvent, was recovered and the average extraction yield calculated using the following formula:

(m / M) x 100, where **m** is the oil mass and **M** the mass of mash. The oil obtained was stored at 4°C before analysis of the profile of fatty acids by gas chromatography.

Fatty acids profile analysis

The crude oil obtained by soxhlet extraction was analyzed by gas chromatography derivatization[15]. The method can be briefly described as follows: We mixed in a glass tube 2 ml of methanol, 0.9 ml of chloroform, 20 microliters of acetic acid, 800 microlitres of MilliQ water, 20 microliters of the crude oil of *Zanthoxylum zanthoxyloides* and 100 microliters of methanol containing the internal standard (C17:0). After 15 min incubation at room temperature, 2 ml of chloroform and 1 ml of MilliQ water were added. The tubes were gently inverted twice and centrifuged 5 min at 1000 rpm to separate the two phases. The lower phase (chloroform) was transferred to another glass tube and a second extraction was done on the first tube with 2 ml of chloroform and the two lower phases were combined. The lipids were dried and hydrolyzed with 400 microliters of KOH (0.5 M) at 100° C for 15 min. Then the fatty acids were transmethylated with 1 ml of boron trifluoride (14% in methanol) at 100° C for 10 min. The FAMEs were then extracted by adding 2 ml of hexane and 1.6 ml of HCl (0.125 M). After homogenization with a vortex, the upper phase was transferred to a GC vial and analyzed by gas chromatography using a Thermo Trace gas chromatograph (Thermo Electron Corporation, Mississauga, ON, Canada). The volume injected was 1 microliter. The injection was made in split mode 1/40 onto a Trace-FAME column (Thermo) with flame ionization type detector. The fatty acids were identified by comparing their retention times with those of authentic standards, and a standard curve was used for quantification[15].

Data analysis

Microsoft Excel was used as data analysis software. The oil extraction yields and percentages of fatty acids are expressed as mean plus or minus standard deviation values.

RESULTS

Oil extracting yield

After performing the extraction three times a yield of 41.8 ± 4.0% was measured.

Fatty acid composition of the crude oil of the seeds of Zanthoxylum zanthoxyloides

We have noted in the crude oil, thirteen measurable fatty acids including six saturated fatty acids and seven unsaturated fatty acids (Figure 2 and Table 1). In terms of proportion, this oil is more concentrated in unsaturated fatty acid (72.35%) than saturated fatty acids (27.65%). The majority unsaturated fatty acids are: oleic acid (46.42 ± 12.65%), linolenic acid (11.73 ± 3.17%) and linoleic acid (12.82 ± 3.46%). The majority of saturated fatty acids are: palmitic acid (23.86 ± 6.40%) and stearic acid (3.27 ± 0.9%) also minority fatty acids in descending order are: vaccenic acid, palmitoleic acid, arachidic acid, lignoceric acid, behenic acid, myristic acid and finally gamma linolenic acid. This oil has a particular fatty acid profile, due to the presence of three essential fatty acids namely linoleic acid (ω6), linolenic acid (ω3) in large quantities and gamma linolenic acid (ω6) in small quantities. Monounsaturated fatty acids represent 47.73% of total fatty acids against 24.62% for polyunsaturated fatty acids.

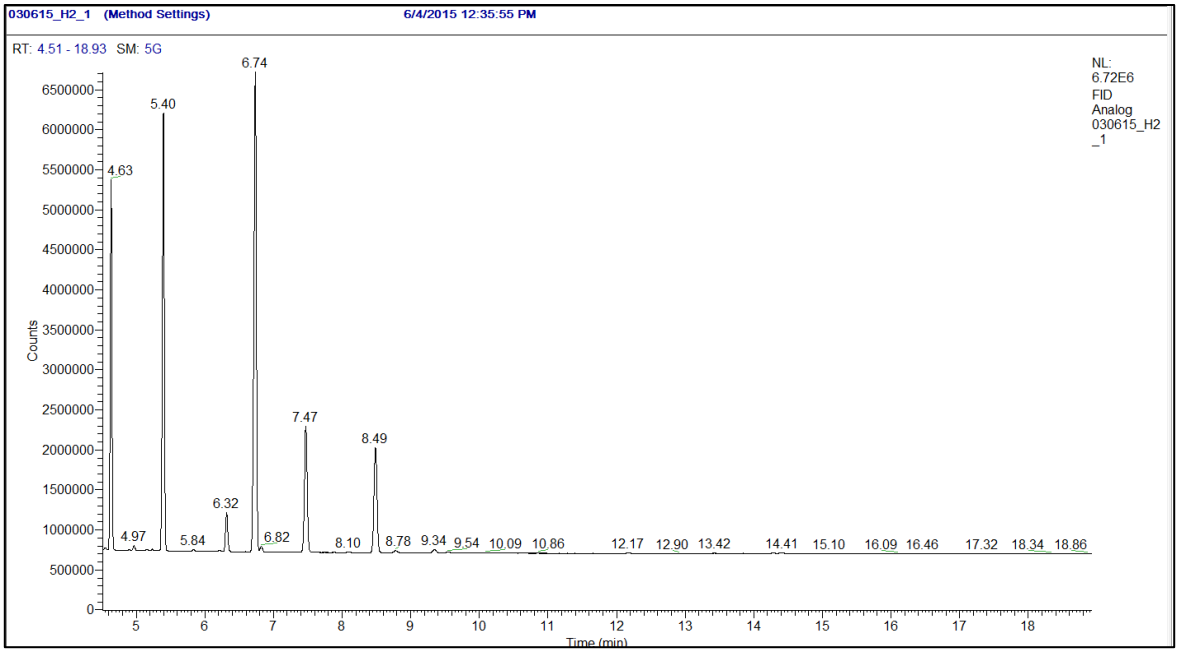

Fig.2: Chromatogram of Zanthoxylum *zanthoxyloides* seeds oil giving retention time and relative abundance of fatty acids

Table 1: Fatty acids of *Zanthoxylum zanthoxyloides seeds oil*

Fatty acid		Percentage (%)	Retention time (min)
C14:0	Myristic acid	0.07 ± 0.02	3.51
C14:1	Myristoleic acid	0.00	-
C16:0	Palmitic acid	23.86 ± 6.4	4.63
C16:1	Palmitoleic acid	0.34 ± 0.09	4.97
C18:0	Stearic acid	3.27 ± 0.9	6.31
C18:1_n-9	Oleic acid	46.42 ± 12.65	6.74
C18:1_n-7	Vaccenic acid	0.53 ± 0.14	6.82
C18:2 n-6	Linoleic acid	12.82 ± 3.46	7.47
C18:3_n-6	Gamma linolenic acid	0.06 ± 0.02	8.10
C18:3_n-3	Alpha linolenic acid	11.73 ± 3.17	8.49
C20:0	Arachidic acid	0.28 ± 0.08	8.78
C18:4_n-3	Stearidonic acid	0.00	-
C20:1	11-eicosenoic acid	0.44 ± 0.10	9.34
C20:2_n-6	11,14-eicosadienoic acid	0.00	-
C20:3_n-6	Gamma-linolenic acid	0.00	-
C20:4_n-6	arachidonic acid	0.00	-
C20:3_n-3	11,14,17-eicosatrienoic acid	0.00	-
C22:0	Behenic acid	0.08 ± 0.02	12.17
C22:1_n-9	Erucic acid	0.00	-
C20:5_n-3	Eicosapentaenoic acid	0.00	-
C22:4_n-6	Docosatetraenoic acid	0.00	-
C24:0	Lignoceric acid	0.09 ± 0.03	16.46
C24:1_n-9	Nervonic acid	0.00	-
C22:5_n-3	Docosapentaenoic acid	0.00	-
C22:6_n-3	Docosahexaenoic acid	0.00	-

DISCUSSION

After extracting the oil in triplicate, we obtained an average oil yield of 41.77 ± 3.98%. This shows that this plant has an interesting oilseed potential. This yield is quite high compared to several common oilseeds as cotton and soya beans contain, respectively, 15-25% and 15-22% oil[9].

The analysis of the profile of fatty acids of the crude oil has allowed us to highlight the presence of six saturated fatty acids and seven unsaturated fatty acids at various concentrations. This oil has a particular fatty acid profile, due to the presence of three essential fatty acids namely linoleic acid (ω6), linolenic acid (ω3) in large quantity and gamma linolenic acid (ω6) in small quantities.

In terms of comparison, peanut oil which is among the most commonly used oils in the region of western Burkina Faso, contains omega-9 and omega-6 fatty acids, which were also measured essential fatty acids in oil of *Zanthoxylum zanthoxyloides*. But peanut oil only contains traces of omega-3 fatty acids[5,4]. Cotton oil is also poor in omega-3 fatty acids, while sesame oils contains both omega-6 and omega-3 fatty acids[5].

In addition the ratio of the proportions of polyunsaturated fatty acids to saturated fatty acids was 0.89, which is slightly above 0.7 considered by some authors as ideal for cooking oil[8]. The ratio of omega-6/omega-3 fatty acids is 1.1 and according to Lambert[5], the maximum ratio of omega-6/omega-3 for edible oil should be 5 thus this ratio for *Zanthxylum zanthoxyloides* oil is well below this maximum value. This ratio is also lower compared to thirteen other edible oils such as grapeseed oil, natural sunflower, cotton, peanut, oleic sunflower, corn germ, safflower, soybean, margarine, primrose, olive, walnut, hemp and rapeseed[5].

Oleic acid (monounsaturated fatty acid) is the major fatty acid measured in *Zanthxylum zanthoxyloides* oil with a proportion of 46.42 ± 12.65% of the total fatty acids, that approximates that of peanut oil[5]. Oleic acid is cited by some authors to have beneficial properties in relation to an extended shelf life and high thermal stability[6] and its elevated concentration in *Zanthxylum zanthoxyloides* oil is a desirable trait.

The proportion of the unsaturated acid is 72.35% of the total fatty acids; which is close to the proportion of unsaturated fatty acids in cotton seed oil[5], with the saturated palmitic acid making up most of the rest of the fatty acid profile as it is the second most important fatty acid in terms of proportion, 23.86 ± 6.40% of total fatty acids. All these results support the use of this oil as food. In addition, the fruits of this species are used as spices in the sauces in some African countries such as Cameroon[1], therefore the seed oil is not the only edible portion of the fruit.

Conclusion and perspectives

This study allowed us to identify the oilseed potential of the seeds of *Zanthoxylum zanthoxyloides* and to specify the fatty acid composition of its oil. These seeds are a rich source of oil which makes up nearly 42% of the dry weight of the seeds. The oil has a particular fatty acid profile because of its high proportion of unsaturated fatty acids (72.35%) with the remarkable presence of oleic acid with a proportion of 46.42 ± 12.65% of the total of fatty acids, and a very desirable omega-6/omega-3 fatty acid ratio of 1.1. Having regard to the profile of these fatty acids, the oil could be used as an edible oil after further studies such as analysis of glycerides, phospholipids and dyes. A cytotoxicity study would also be warranted. Once these studies are successful, we will consider an extension of the production of this plant as an oilseed plant.

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