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Lipid content and fatty acid profile of the fruit seeds of *Diospyros mespiliformis*

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Abstract

The lipid content and profile of oil from *Diospyros mespiliformis* seeds was determined. Total oil content of the seeds was low at 0.70 ± 0.17 %. Although the oil yield was low, the fatty acid profile shows an interesting pattern: split as 39.54% total saturated fatty acids with palmitic acid (C16:0) at 30.06 ± 0.61 % of the total saturated fatty acids: total monounsaturated fatty acids constituted 29.42% of the oil yield with palmitoleic acid (C16:1n7) at 29.37 ± 0.38 % making up the large proportion of the monounsaturated fatty acids. Total polyunsaturated fatty acids grossed 29.66% of the oil yield and were divided as 28.71 ± 1.79 % linoleic (LA, C18:2n6) acid and 0.95 ± 0.61 % α -linolenic (ALA, C18:3n3) acid. In terms of fatty acid profile, *D. mespiliformis* seed oil has a high potential as a source of LA and ALA, both essential fatty acids. However a lot of breeding work has to be invested into the tree in order to come up with provenances of *D. mespiliformis* with higher oil yields to warrant commercial exploitation of its fruit kernel oil.

Keywords: *Diospyros mespiliformis*, seeds, lipid profiles, linoleic and α -linolenic acids.

INTRODUCTION

Non-cereal plant foods in Southern Africa and most of the developing tropical world contribute significantly to the diets of indigenous rural communities, especially during periods of grain shortages (Schreckenber *et al.*, 2006). The indigenous trees, apart from contributing to fuel-wood and timber products, also supply non-timber products in the form of fruits, nuts and honey which help communities survive famine in lean agricultural years. Indigenous fruit trees, for example, *Parinari curatelifolia*, *Uapaca kirkiana*, *Adonsia digitata*, *Mimusops zeyheri* and *Diospyros mespiliformis* which are sources of edible fruit rich in vitamins and minerals, play a crucial role in poverty reduction in rural communities (Garrity, 2004; Russel and Franzel, 2004).

The Jackal-berry tree *D. mespiliformis* (family Ebenaceae) grows in tropical areas of Africa stretching from Sudan, where its fruit pulp is used to make a fermented drink, to South Africa (Dalziel, 1937). The tree is largely found in riverine areas and on anthills

where soils are relatively fertile. It has white fragrant flowers (Kerharo, 1974). The fruit is globose, fleshy, upto 3cm in diameter, generally greenish and pubescent when young, yellowish to orange yellow when ripe. Each ripe fruit contain 4–6 dark brown bean-shaped seeds. The soft and sweet pulp of its fruit is eaten by a host of wild animals and humans while the tree's leaves and soft twigs provide fodder for wildlife and is largely browsed by giraffe and elephants.

Various parts of the tree *D. mespiliformis* are reported to have immense ethnomedicinal value. Extracts from its leaves and bark are used to treat a wide array of diseases (Belemtougri *et al.*, 2006). A leaf decoction is used in the treatment of sleeping sickness, malaria and headache (Kerharo, 1974, Etkin, 1997). Additionally the same decoction is used as an antihelmintic (Kerharo, 1974; Khan *et al.*, 1980). Nwude and Ebong (1980) report the bark as providing good relief for coughing. Sanogo *et al.* (1998) indicated that the water and methanol extracts of the leaves exhibited antibacterial effects yet the chloroform and petroleum extracts did not. The plant has also been used to enhance recovery from pain and fever (Adzu *et al.*, 2002a). Root bark extracts from *D. mespiliformis* have been shown to contain diosquinone, a naphthoquinone epoxide, observed to be very active against a number of cancer cell lines (Adeniyi *et al.*, 2003).

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Alkaloids, saponins, quinones, sterol, tannins and triterpenes found in the stem bark extract of the tree (Delaveau *et al.*, 1979, Zhong *et al.*, 1984) are thought to be some of the compounds that contribute to the plant's array of uses in ethnomedicine. In their studies on the central nervous activity of the plant extracts in animals, Adzu *et al.* (2002b) indicated that the aqueous extracts of *D. mespiliformis* stem bark caused a significant prolongation of the pentobarbital-induced sleeping time, reduced spontaneous motor activity (SMA) and exploratory behaviour in mice. Adzu *et al.* (2002b) also noted that although the stem bark extracts prolonged the onset of the phases of seizure activity in mice, they did not protect them against lethality induced by pentylenetetrazole over and above failing to affect the motor coordination test. From their studies Adzu *et al.* (2002b) concluded that the extract from the stem bark of *D. mespiliformis* contains an entity with neuropharmacological activity which could be sedative in nature.

Most of the research on *D. mespiliformis* has centered on the medicinal value of leaf and stem bark extracts, however, the fruit nuts/kernels of many plants have also been shown to contain essential and non-essential oils and compounds with beneficial pharmacological and/or industrial uses. The aim of the current study is to profile the lipid fraction of *D. mespiliformis* fruit kernels, with a view to determine their potential as a source of essential fatty acids for culinary, cosmeceutical, medicinal, dietetics and, or pharmacological exploitation.

MATERIALS AND METHODS

Reagents used to extract the lipids and for the fatty acid methyl ester preparation were obtained from Merck Chemicals, South Africa.

Plant Source

The fruit of the plant *D. mespiliformis* was collected from the Mandamabwe area of Chivi District in Zimbabwe, latitude 20°67'S, longitude 30°56'E and altitude 693m above sea level. The area is characterized by mean maximum annual ambient temperatures of 30°C and a mean annual rainfall of 535mm (Magorokosho *et al.*, 2007). Tree samples (branches, fruit and seeds) were submitted to the National Botanical Gardens of Zimbabwe who confirmed the field identification of the tree. Ten trees from the site were randomly sampled for their fruit and the seeds contained therein were used in the lipid and fatty acid assays. A hundred ripe fruits were collected from each tree. Out of the 100 fruits from each tree, 10 fruits were randomly selected and their seeds were used. Extracted seeds (from each of the selected 10 fruits) were sun dried for two weeks in the shade and stored under room

temperature in an airtight container in a cabinet pending analysis. Just prior to analyses the seeds were mixed and crushed in a blender to generate a composite sample. The composite sample was divided into three equal portions that were analyzed in parallel.

Dry matter determination

Samples, in triplicate, of the crushed *D. mespiliformis* kernels were dried in an oven at 60°C to constant mass (after 6 days).

Lipid extraction and profiling

Standard procedures were used for lipid extraction (Bligh and Dyer, 1959). In summary, 10g of sample was mixed and blended in 100ml chloroform-methanol mixture (2:1) and left to extract overnight at 4°C. The samples were then filtered through filter paper (Whatmann No.1, size 18 mm) and 30ml 0.9% saline added, mixed, and allowed to stand overnight at 4°C to allow separation into two phases. The bottom (chloroform) phase was collected and reduced to dryness under vacuum at 37°C and then made up to 20ml with chloroform and stored at -20°C for future analysis. Methyl esters of the fatty acids were prepared using 10% acetyl chloride in methanol with incubation at 50°C overnight. The methyl esters were extracted into hexane. They were separated on a Varian 3400 gas chromatograph isothermally at 195°C with a 10% SP2330 on chromosorb 100/120 WAW 2 m × 3.2 mm column and quantitated using FID and a Varian 4270 integrator. Peaks were identified by comparison with authentic fatty acid standards. The lipid and fatty acid standards were obtained from Sigma Aldrich. Data is expressed as mean ± S.D.

RESULTS

Dry matter and oil content

The dried seeds had a mean dry mass of 90.33 ± 0.46% and a mean oil content of 0.70 ± 0.17%.

Lipid profile

The lipid profile of crushed *D. mespiliformis* seeds is shown in Table 1. Saturated fatty acids (SFA) constituted 39.54% of the lipid fraction of the seeds with palmitic acid (C16:0) at 30.06 ± 0.61 % making up the bulk of the SFA. Palmitoleic acid (C16:1n7) was the dominant monounsaturated fatty acid (MUFA) at 29.37 ± 0.38% out of a total of 29.42% of the MUFAs. Two polyunsaturated fatty acids (PUFAs) were noted: linoleic acid (C18:2n6) and α -linolenic acid (C18:3n3) at 28.71 ± 1.79% and 0.95 ± 0.61%, respectively.

DISCUSSION

The seeds were difficult to work with. They could not be easily ground in a blender and had to be hammer-crushed before grinding in a blender.

Oil content

The oil content of the seeds from *D. mespiliformis* (0.7±0.17 %) was much lower than previously observed in other indigenous tree fruit nuts/kernels, for example, *Ximenia caffra* and *Ricinodendron rautanenii*, (47.6±7.5% and 53.3±13.7%, respectively) (Chivandi *et al.*, 2008) from the same general eco-environment in Zimbabwe. The oil yield was also lower than that from traditional oil plants such as, soyabean (*Glycine max*) and cotton seed (*Gossipium hirstum*), with mean oil yield of 15–25% and 35–40%, respectively (Manga *et al.*, 2000). The very low oil content from the seeds of *D. mespiliformis* could be due to the probable high content of other organic entities that could largely constitute the mass of the seed. Delaveau *et al.* (1979) and Zhong *et al.* (1980) noted that stem bark extracts of *D. mespiliformis* contained a high concentration of organic entities especially alkalioids, saponins, quinones, sterols, tannins and triterpenes. It could well be that the same entities are more concentrated in the seed whose lipid extract had a “sharp” green-yellowish color, a possible indicator of other “organics” that were extracted together with the lipid fraction. The envisaged high concentration of the other organic entities could be the reason for the very low oil content of seeds of *D. mespiliformis*.

Fatty acid profiles: potential and associated risk

Linoleic acid (LA; C18:2n6) and α -linolenic acid (ALA, C18:3n3) reported in the *D. mespiliformis* seed oil are essential fatty acids that are vital in the maintenance of some key physiological functions of the animal body. Linoleic acid is essential for maintaining the integrity of the skin, cell membranes, the immune system, and for synthesis of icosanoids (Dupont *et al.*, 1990). Icosanoids are vital for reproductive, cardiovascular, renal, and gastrointestinal functions and resistance to disease (Dupont *et al.*, 1990). Linoleic acid constituted 28% out of the 29% PUFA content of *D. mespiliformis* (Table 1). If use of molecular breeding techniques could be harnessed to generate *D. mespiliformis* provenances with commercial viable oil yields, the seed oil, due to the expressed high levels of LA, could be valuable as a dietary supplement, for the de novo synthesis of icosanoids. The latter (icosanoids derived from LA) would help mitigate against dysfunction of the cardiovascular, renal, reproductive, and gastrointestinal and immune systems. ALA, which constituted about 1% of the *D. mespiliformis* seed oil (Table 1), is the parent molecule of the Omega-3 essential fatty acid family and is converted in the body

Table 1: Mean fatty acid profiles of *D. mespiliformis* seed oil

Fatty Acid	Fatty Acid Content (%)
Saturated	
C14:0 (myristic acid)	0.62 ± 0.04
C16:0 (palmitic acid)	30.06 ± 0.61
C18:0 (stearic acid)	7.74 ± 0.44
C20:0 (arachidic acid)	1.12
Total Saturated Fatty Acids	39.54
Monounsaturated	
C14:1n7 (myristoleic acid)	0.05
C16:1n7 (palmitoleic acid)	29.37 ± 0.38
Total Monounsaturated Fatty Acids	29.42
Polyunsaturated Fatty Acids (n6PUFA)	
C18:2n6 (linoleic acid)	28.71 ± 1.79
Polyunsaturated Fatty Acids (n3PUFA)	
C18:3n3 (α -linolenic acid)	0.95 ± 0.61
Total Polyunsaturated Fatty Acids	29.66
TPUFA: TSFA	0.75 : 1
n6PUFA : n3PUFA	30.22 : 1

to two other members of the Omega-3 group: docosahexaenoic acid (DHA, C22:6n-3) and eicosapentaenoic acid (EPA, C20:5n-3). Decreased tissue levels of DHA (C22:6n-3) are implicated in the etiologies of non-puerperal and postpartum depression (Levant *et al.*, 2008). Levant *et al.* (2008) report that in their studies rats that received a diet with adequate levels of ALA did not display neurobiological alterations attributable to decreased brain DHA while those on the test diet (had decreased brain DHA) exhibited signs associated with decreased hippocampal brain-derived neurotrophic factor (BDNF) gene expression and increased relative corticosterone response to an intense stressor. In virgin female rats with decreased brain DHA, serotonin content and turnover in frontal cortex were decreased compared to virgin female rats with normal brain DHA (Levant *et al.*, 2008). *D. mespiliformis* seed oil has potential to be used as a source of metabolic raw material for DHA in the form of ALA. The latter (ALA) in the seed oil can be used as a dietary supplement to arrest the development and progression of DHA deficiency triggered depression.

Clinical studies clearly point out that the ingested ratio of n-6 to n-3, particularly LA versus ALA, as an important determinant to the maintenance of cardiovascular health (Okuyama, 2001; Simopoulos, 2003; Griffin, 2008). Metabolites of n-6 are more inflammatory than those of n-3 fatty acids. This necessitates that n-3 and n6 fatty acids be consumed in a balanced proportion. Healthy ratios of n-6:n-3 are reported to range from 1:1 to 4:1. The LA (n-6): ALA (n-3) ratio (30.22:1) of the *Diospyros mespiliformis* seed oil (Table 1) is much larger and way outside the recommended range. Use of the oil would, therefore, demand its fractionation to its components so as to

avoid the intake of the disproportionate LA: ALA ratio that would lead to metabolic upsets associated with increased risk and incidences of cardiovascular disease.

Low oil yield: the challenge

Understanding the level, structure and origin of genetic variation within and among populations of tropical trees is essential for devising optimum management strategies for their sustainable utilization and conservation (Russell *et al.*, 2002). This is essential in view of the potential of the *D. mespiliiformis* seed oil as a source of two essential PUFAs, LA and ALA, that have tremendous applications in the medical field. The oil yield from the seeds is so low that it militates against the potential as expressed in the fatty acid profile. A lot of work, using high technology molecular breeding techniques, needs to be dedicated towards screening and breeding provenances of the *D. mespiliiformis* trees, that have a higher kernel oil yield for it to be commercially exploited. This is critical since it is important to determine if the *D. mespiliiformis* oil yield and LA and ALA profiles (marketable traits) are amenable to genetic improvement (Leakey and Simons, 1997).

CONCLUSION

The current very low oil yield from *D. mespiliiformis* seeds does not lend it for commercial exploitation in spite of its potential as a source of both LA and ALA. Any future research has to focus on the genetic (molecular breeding techniques) improvement of the oil yield and maintenance of the LA and ALA profiles in the seeds of *D. mespiliiformis* fruit seeds.

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