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Physicochemical characterisation of hexanic seed oil extract from the pepper tree (*Schinus molle*) of South African origin

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The physicochemical characterisation of oil extracts from *Schinus molle* seeds collected in South Africa was performed. The oils were extracted in hexane, physicochemical parameters determined and lipids profiled by gas chromatography, in order to determine its potential for use in industry, ethnomedicine and its nutritional value. The total oil yield of the seed dry mass was 22%. The oil was semi-liquid at room temperature and consisted of 24 fatty acids of which 15.56, 16.75, and 31.02% were saturated, monounsaturated and polyunsaturated fatty acids, respectively. Palmitic acid (8.31%), oleic acid (15.3%) and linoleic acid (26.99%) were the main fatty acids in *S. molle* seed oil, which had a high acid value (178.23 ± 36.8 mg KOH/g), iodine value (17.74 g I₂/100 g oil) and saponification value (129.88 mg KOH/g). It was concluded that oil from *S. molle* seeds could be used as a source of palmitic, oleic and linoleic acid which would be utilised as industrial ingredients in the manufacture of soaps, pharmaceutical products, cosmetics and nutritional supplements.

Key words: *Schinus molle*, hexane oil extraction, fatty acids, ethnomedicine.

INTRODUCTION

The pepper tree, *Schinus molle* belongs to the family Anacardiaceae, which comprises of more than 30 tree and shrub species that are native to South and Central America (Barkley, 1994; Mabberley, 1990). The *S. molle*

tree has been cultivated in Southern African countries including Botswana, Zambia, Mozambique, Malawi, South Africa and Zimbabwe (van der Lingen, 1930; Coates-Palgrave, 1988). The pepper tree, often referred to as "piral" or "chile" in Costa Rica, is a resinous evergreen small tree which grows up to 7 to 10 m and has sparse pinnate compound, long, thin leaves (Gupta, 1995; Holdridge et al., 1997). Its edible ripe fruit is about 5 mm in diameter and often pink reddish to red in colour.

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Indigenous communities in the subtropical climates use the pepper tree for landscaping, windbreaks, river bank protection and watershed conservation (Benzi et al., 2009; Deveci et al., 2010).

The *S. molle* tree is used in ethnomedicine by indigenous people in the tropics due to its analgesic, antimicrobial, cytotoxic, anti-rheumatic, anti-septic, anti-inflammatory and anti-fungal properties (Gupta, 1995; Barrachina et al., 1997; Ruffa et al., 2002; Yueqin et al., 2003; Diaz et al., 2008; Deveci et al., 2010). Previous studies have shown that essential oil extracts from leaves and fruits of *S. molle* can be used as a repellent against the oriental cockroach, *Blatta orientalis* (Deveci et al., 2010), khapra beetle, *Trogoderma granarium* and red flour beetle, *Tribolium castaneum* (Abdel-Sattar et al., 2010). *S. molle* leaf essential oil extracts have also been used as an insecticide for the Chagas disease vector, *Triatoma infestans* (Ferrero et al., 2006).

The use of natural plant products such as *S. molle* is gaining prominence in ethnomedicine as practitioners seek substitutes for synthetic pharmaceutical products. Most synthetic pharmaceutical products are believed to possess several physiological side effects, hence the increased usage of natural plant products. Natural products from trees and shrubs both indigenous and exotic to Africa are not fully exploited for their nutritional, industrial and medicinal potential because the physicochemical characteristics are unknown. The fruit is used for producing red pepper, while the seed is often thrown away after utilisation of the fruit pulp (Cremonini, 1928). Several studies have focused on the *S. molle* leaf oil extracts (Montes et al., 1961; Jennings and Bernhard, 1975; Dikshit et al., 1986; Rossini et al., 1996; Abdel-Sattar et al., 2010; Ennigrou et al., 2011), but there is a dearth of information on the *S. molle* seed oil.

The current study was aimed at determining the oil content, fatty acid profile and physicochemical properties of the *S. molle* seed oil extract, in order to explore the industrial, domestic and medicinal potential.

MATERIALS AND METHODS

Seed collection and identification

The *S. molle* ripe fruits, from which seeds were extracted, were randomly collected from trees growing in the vicinity of the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa (satellite location S26° 10' 38.4" E28° 02' 34.1" Elevation 1770 m). The seeds were transported to the Department of Pharmacology, University of the Witwatersrand, Johannesburg, South Africa and were identified by a plant taxonomist in the School of Animal Plant and Environmental Science at the University of the Witwatersrand, Johannesburg, South Africa. They were air-dried at room temperature and stored in air-tight containers to prevent aerial oxidation.

Determination of oil yield

The composite sample of seeds from all the trees were dried and weighed to obtain their dry mass and crushed using a mortar and

pestle. Standard procedures were used for the oil extraction (Akubugwo and Ugboogu, 2007; Deveci et al., 2010). Briefly, the crushed seeds (258.2 g) were transferred into a bottle with 950 ml of hexane (Merck Chemicals, Wadeville, South Africa). The bottle was covered by plastic wrap (Parafilm M®; Pechinery Plastic Packaging, Illinois, United States of America) and shaken continuously for five days. The sample was filtered (Whatmann No.1, size 18 mm, Lasec, South Africa, Johannesburg) and the hexane evaporated to obtain the oil which was then placed into 5 ml vials before further analysis. The yield of the extracted oil (%) was expressed on a dry matter basis.

Fatty acid characterisation

The extracted *S. molle* oil sample (5 ml) was sent to the Agricultural Research Council's Irene Analytical Services, Pretoria, South Africa for lipid analysis using gas chromatography as previously described (Christopherson and Glass, 1969; El-Beltagi et al., 2007). Briefly, the oil extract was transmethylated with 2 M methanol-sodium hydroxide. The resulting fatty acid methyl esters were then extracted in heptane, filtered and dried under nitrogen. A gas chromatogram (HP6890 GC, Hewlett Packard, Bristol, UK) with a BB-23 capillary column (90 cm x 250 µm x 0.25 µm) (Supelco, Sigma-Aldrich, Johannesburg, SA) and a flame ionisation detector was used to separate the fatty acids. Detector and injector temperatures were set at 300°C. A computer installed with CHEMSTATION software (Chemstation, Deutschland GmbH, Augastrasse, Germany) was used to quantify the fatty acids. Nonadecanoic acid was used as an internal standard.

Determination of iodine value

Iodine value (IV) was determined using the iodine monochloride method previously described (Brisbois et al., 2004). Briefly, 1.02 g of the oil extract was dissolved in 10 ml carbon tetrachloride (Merck Chemicals, Wadeville, South Africa) in a 100 ml flask. 25 ml of the iodine monochloride solution was added to the oil sample and allowed to stand in a dark cupboard for 2 h at a room temperature of 25°C. After 30 min, 20 ml of 10% potassium iodide solution were added and the mixture titrated against 0.1 N sodium thiosulphate using starch mucilage, as an indicator. The amount of sodium thiosulphate (a) required to titrate the mixture up to the end point was recorded. A blank determination was also performed in the same manner but without the seed oil and the amount of sodium thiosulphate (b) required to titrate the mixture up to the end point was recorded. The IV determinations were done in duplicate and calculated using the formula:

$$\text{Iodine value (IV)} = \frac{(b - a) \times 0.01269 \times 100}{\text{Weight of oil sample in grams}}$$

Determination of saponification value

Saponification value (SV) was determined by dissolving 2.03 g of the oil extract in 12.5 ml of 0.5% ethanolic potassium hydroxide (Merck Chemicals, Wadeville, South Africa) in a 100 ml flask. The mixture was refluxed in a reflux condenser (Schott Technical Glass Solutions GmbH, Germany) for 30 min and 1 ml of phenolphthalein indicator (Techno Pharmachem, India) was added thereafter. The hot soap mixture was titrated against 0.5 N hydrochloric acid until end-point was reached and the amount of hydrochloric acid required (a) was recorded. A blank determination was also performed in the same manner but without the seed oil being tested and the amount of 0.5 N hydrochloric acid (b) required to titrate the

Table 1. Physical and chemical properties of *S. molle* seed oil extracts.

Property	Observation/ value
Percentage oil yield	22%
Colour	Greenish-yellow
Odour	Menthol-like
State at 25°C	Semi-liquid
Iodine value	17.74 g I ₂ /100 g of oil
Saponification value	129.88 mg KOH/ g of oil
Acid value	178.23 ± 36.80 mg KOH/ g of oil

mixture up to the end point was recorded. The SV was done in duplicate and was calculated using the formula:

$$\text{Saponification value (SV)} = \frac{(b - a) \times 0.02805 \times 1000}{\text{Weight of oil sample in grams}}$$

Determination of acid value

Acid value (AV) was determined based on the acid-base titration in non-aqueous solvent as previously described (International Standards Organisation 660, 1983). Briefly, 1.05 g of the oil extract was dissolved in 5 ml of 1:1 v/v ethanol: diethyl ether solvent in a 100 ml flask and heated until the oil had completely dissolved. The mixture was then titrated against 0.1 N aqueous sodium hydroxide using 1 ml phenolphthalein as an indicator (Techno Pharmachem, India), shaken constantly, until a pink colour which lasted for 15 s was obtained. The amount of sodium hydroxide required (a) to titrate the mixture up to the end point was recorded. Acid value determinations were done in duplicate and AV calculated as follows:

$$\text{Acid value (AV)} = \frac{a \times 0.00561 \times 1000}{\text{Weight of oil sample in grams}}$$

RESULTS AND DISCUSSION

The physical and chemical characteristics of *S. molle* seed oil extract are summarised in Table 1. The oil yield was 22% on a dry matter basis and this is comparable to oil yield from soy bean seeds, *Glycine max* (15 to 25%) (Cheftel and Cheftel, 1977) and cotton seed, *Gossypium hirsutum* (18 to 26 %) (Jones and King, 1996). The yield from this oil is appreciably high and possible commercial extraction is worthwhile.

S. molle seed oil had an iodine value of 17.74 I₂/100 g of oil (Table 1). Iodine value is an index of the degree of unsaturation of oil (Pocklington, 1990). The higher the iodine value, the less stable the oil and the more vulnerable to oxidation and free radical production. *S. molle* seed oil iodine value is comparable to that of palm kernel seed oil (*Elaeis guineensis*); whose iodine value is between 13 to 17 I₂/100 g of oil (Chong and Siew, 1994). This means that *S. molle* seed oil is non-drying and

saturated like coconut; *Cocos nucifera* seed oil (Richardson, 1911) and palm kernel seed oil. Saturated oils with low iodine value can be classified as non-drying oils since they are not vulnerable to oxidation or polymerisation when exposed to the atmosphere and they tend to thicken to form a hard dry film (Essien et al., 2012). The drying property of the *S. molle* seed oil makes it a potential candidate as a base for the manufacture of paints and varnishes. Unlike soy bean seed oil whose oxidative stability is low due to its high iodine value (130 I₂/ 100 g of oil), the high oxidative stability of the *S. molle* seed oil makes it safe for cooking and use as a dietary supplement.

S. molle seed oil saponification value was 129.88 mg KOH/g oil. The saponification value for the *S. molle* seed oil was lower than that of white-seed melon (*Cucumeropsis mannii*) (220.19 mg KOH/g) and palm oil (*E. guineensis*) (196 to 205 mg KOH/g) (Folkard and Sutherland, 1996; Essien et al., 2012). Oils with high saponification value are used as ingredients in soap making, and manufacture of lather shaving cream and cosmetics (Thomas, 2002; Nzikou et al., 2007). The *S. molle* seed oil had an acid value of 178.23 ± 36.8 mg KOH/g oil. Acid value of oil is the mass of KOH that is required to neutralise 1 g of vegetable oil and it reflects the quality of oil from plant material and its oxidative stability (Kuselman and Shenhar, 1997; Kardash and Tur'yan, 2005). *S. molle* seed oil had an unusually high acid value compared to cotton seed oil (0.65 mg KOH/g oil), white-seed melon (7.09 mg KOH/g oil) and soy bean oil (0.67 mg KOH/g oil) (Khan et al., 2001; Essien et al., 2012). The unusually high value may possibly be due to the presence of polyphenols in the shells of the seeds since the seeds were not shelled when they were crushed. It has been suggested that oils with low acid value exhibit high oxidative stability and increased shelf life and may be used in the industrial production of oil-based paints (Essien et al., 2012).

The fatty acid profile of *S. molle* seed oil extract is summarised in Table 2. The total yield of the saturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids was 15.56, 16.75 and 31.02%, respectively. 36.59% of the fatty acids were quantified but could not be identified. The major saturated fatty acids in *S. molle* seed oil were palmitic acid (8.31%) and stearic acid (2.71%). Stearic acid and palmitic acid are primarily found in natural fats and oils (Emken, 1994) and their presence in appreciable amounts in the *S. molle* seed oil means that this oil may potentially be used as a pharmacologically inactive carrier for active ingredients in the manufacture of pharmaceutical products. The high viscosity and stability of stearic acid in *S. molle* seed oil makes it a good industrial lubricant for preventing ingredients from clumping together during drug manufacture (Tseng et al., 1999). Although the quantity of stearic acid is small relative to other fatty acids, its presence in *S. molle* seed oil means that this oil can be used as an ingredient for candle, plastic and cosmetic

Table 2. Fatty acid profile of the pepper tree, *Schinus molle* seed oil extract.

Fatty acid	Yield of seed oil (%)
Saturated (SFA)	
C8:0 (Caprylic)	0.91
C10:0 (Capric)	0.17
C11:0 (Undecylic)	0.25
C12:0 (Lauric acid)	0.5
C14:0 (Myristic acid)	0.68
C16:0 (Palmitic acid)	8.31
C17:0 (Margaric acid)	0.26
C18:0 (Stearic acid)	2.71
C20:0 (Arachidic acid)	0.87
C21:0 (Heneicosanoic acid)	0.11
C22:0 (Behenic acid)	0.50
C24:0 (Lignoceric acid)	0.29
Total saturated fatty acids (TSFA)	15.56
Monounsaturated (MUFA)	
C14:1 (Myristoleic acid)	0.18
C16:1 (Palmitoleic acid)	0.28
C18:1n9c (Oleic acid)	15.30
C22:1n9 (Erucic acid)	0.31
C24:1 (Nervonic acid)	0.41
C20:1 (<i>cis</i> -11-eicosenoic acid)	0.27
Total monounsaturated fatty acids (TMUFA)	16.75
Polyunsaturated (PUFA)	
C18:2n6c (Linoleic acid)	26.99
C18:3n3 (α -linolenic acid)	2.78
C18:3n6 (γ -linolenic acid)	0.13
C20:2 (<i>cis</i> -11,14-eicosadienoic acid)	0.18
C18:1n9t (Eliadic acid)	0.13
C20:5n3 (<i>cis</i> 5,8,11,14,17-Eicosapentenoic acid)	0.82
Total polyunsaturated fatty acids (TPUFA)	31.02
Trans fatty acids	0.13
Cis fatty acids	42.3
Unknown	36.59
EPA (Eicosapentaenoic acid) (20:5n-3)	0.82
Omega 3	3.59
Omega 6	27.12
Omega 9	15.75
TPUFA:TSFA	2

manufacture. While palmitic acid has been shown to possess a cholesterolaemic effect in relation to other dietary fatty acids (French et al., 2002), a study using a rodent model has shown palmitic acid possesses anti-oxidant properties that help prevent the development of atherosclerosis (Cho et al., 2010). The utilisation of *S. molle* as a source of palmitic acid and steric acid for medicinal use require further investigation.

The *S. molle* seed oil extract had 15% of the monoun-

saturated fatty acids oleic acid which is comparable with the seed oil content from an indigenous Southern African tree, *Kigelia africana* of 18% (Chivandi et al., 2011). Oleic acid is predominantly found in plant products and is a good precursor of Omega 9 unsaturated fatty acids. It is physiologically relevant for its ability to lower blood pressure (Terés et al., 2008) and levels of low density lipoprotein in the body (Aviram and Elias, 1993). Oleic acid is also an anti-oxidant that assists in scavenging free

radicals and boosting the immune system by minimising cellular damage caused from free radicals (Cho et al., 2010). Studies have also shown that oleic acid possesses anti-cancer activities and has anti-inflammatory effects (Vassiliou et al., 2009), and may improve joint pain in arthritic patients. The presence of significant quantities of oleic acid in *S. molle* seed oil makes it a potential antioxidant and nutritional supplement, though further studies to confirm this are required.

Linoleic acid constituted 26.99% of the extracted fatty acids from the *S. molle* seed oil and was the major polyunsaturated fatty acid. Linoleic acid is a precursor of omega 6 polyunsaturated fatty acids and is commonly found in dietary plant oils such as grape seed oil, sunflower oil and soy bean oil (Kris-Etherton et al., 2002). It is vital for balancing essential plasma fatty acid ratios which is important for weight loss and maintaining healthy bodies, especially with the rising prevalence of obesity and metabolic syndrome that have reached epidemic levels and have become a major global health problem (Spalding et al., 2009).

The *S. molle* seed oil had an unsaturated to saturated (the total yield of the polyunsaturated fatty acids to the total yield of the saturated fatty acids) ratio of 2 indicating that the oil had more unsaturated fatty acids than the total saturated fatty acids. This is comparable with the root extracts from *Nyctanthes arbortristis* whose total yield of the polyunsaturated fatty acids to the total yield of the saturated fatty acids ratio was 2.1 (Rahman and Shajahan, 2011). Human food containing significant quantities of polyunsaturated fatty acids are widely considered to be beneficial to health, particularly reduction of the risk to develop cardiovascular diseases (Mozaffarian et al., 2005). Future studies should focus on the use of appropriate *in vitro* and *in vivo* animal models to assess the feasibility of using *S. molle* as an anti-cancer, anti-inflammatory and anti-oxidant agent.

The present study has shown that the physicochemical properties and constituent fatty acids from *S. molle* seed oil extracts have the potential for use in industry, ethnomedicine and as a nutritional supplement. The potential of *S. molle* seed oil as source of oleic acid and linoleic acid in cardiovascular and other disease management need further investigation.

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