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Fatty Acid Composition and Antimicrobial Activity of *Baphia massaiensis* Seed Oil

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Authors' contributions

This work was carried out in collaboration between all authors. Author NK designed the study, performed the study under the supervision of authors RRTM and IM. Author OM wrote the manuscript and data analysis. All authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

Aims: The seed oil composition of *Baphia massaiensis* seeds was determined using ^{1}H NMR and GC-MS techniques. The seed oil was also screened for antimicrobial activity.
Study Design: The study was designed to determine *Baphia massaiensis* seed oil fatty acid composition and antimicrobial activity.
Place and Duration of Study: Department of Chemistry, University of Botswana, between June 2012 and July 2014.
Methodology: The *Baphia massaiensis* seed cotyledons powder (29.2 g) were extracted by Soxhlet extraction using n-hexane/ 1-propanol. The seed oil (3.12 g) was esterified to FAMEs using dry methanol. The percentage composition of fatty acids methyl esters (FAMEs) in the seed oil of *B. massaiensis* was determined using ^{1}H NMR and GC-MS techniques. The antimicrobial screening was carried out using agar well diffusion method.
Results: The ^{1}H NMR method showed the oil composition to be 20% ω-3 fatty acids, 11% mono-unsaturated, 60% di-unsaturated and 9% saturated fatty acids. Based on GC-MC analysis,

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saturated fatty acids composition was 12.2% and unsaturated fatty acids were 87.8% of the total FAMEs. The major constituents of *B. massaiensis* seed oil FAMEs were linoleic acid ($C_{18:2}$; 49.0%) and linolenic acid ($C_{18:3}$; 36.7%) methyl esters. The FAMEs were active against *E. coli, S. aureus* and *B. subtilis* with 10-16 mm inhibition zones.

Conclusion: Linoleic acid (49.0%) and linolenic (36.7%) methyl esters were the major components of *Baphia massaiensis* FAMEs.

Keywords: Baphia massaiensis; seed oil; fatty acid methyl esters; ^{1}H-NMR; GC-MS; linoleic.

1. INTRODUCTION

Plant seed oils are commonly used as flavourants in food, perfumeries, pharmaceutics and cosmetic industries due to their attractive biological and pharmacological properties. They are also used as sources of biodiesel [1]. Therefore there is a growing need to explore plant seed oils of non-economical plants for possible application in food and fuel industry [2,3]. There are various methods for identifying the fatty acid composition of plants. Among them Gas Chromatography Mass spectrometry (GC-MS) and Nuclear Magnetic Resonance (NMR) are commonly used techniques to determine the composition of the non-polar extracts and volatile oils. Chromatographic analysis of Fatty Acid Methyl Esters (FAMEs) is an important tool in the characterization of fats and oils, and in the determination of total fatty acids, and unsaturated fatty acids content in foods [4]. GC-MS, is a simple, rapid and relatively inexpensive method for the identification and quantification of FAMEs in lipid research [5].

Baphia massaiensis belongs to the family Fabaceae (Leguminoseae), sub-family *Faboideae* (Papilionaeae) and tribe *Sophoreae*. It is used for medicine, general health upkeep and for ornamental purposes. Its traditional uses in treatment of sores, wounds and hemorrhages imply that it possesses antimicrobial, wound healing and anti-inflammatory properties. To the best of our knowledge, there are no reports about the seed oil fatty acid composition of *Baphia massaiensis*. Hence, our interest in its extraction, characterization by ^{1}H NMR and GC-MS.

2. MATERIALS AND METHODS

2.1 General Methods

All solvents used were of analytical grade. Esterification reaction was monitored by Thin-Layer Chromatography (TLC) on aluminium sheets (Silica gel $60F_{254}$, layer thickness 0.2 mm) from Merck. Infrared spectra were recorded neat on a Perkin Elmer FT-IR spectrophotometer 1000 (4000-600 cm^{-1}). ^{1}H NMR spectrum was recorded on a Bruker Avance DPX 300 MHz NMR spectrometer in $CDCl_3$ with tetra-methyl silane (TMS) as an internal standard at room temperature. Chemical shifts (δ) were measured in ppm relative to internal TMS standard.

2.2 The Preparation of Methyl Esters of Fatty Acids

The *Baphia massaiensis* seed cotyledons powder (29.2 g) were placed into a thimble in a Soxhlet chamber with a siphon arm. Then Soxhlet extraction procedure using n-hexane/ 1-propanol (3:1 v/v) following methods defined in literature was adopted [6-9]. The Soxhlet extraction was carried-out for 8 hours, after which the solvent was filtered and concentrated in vacuo. The seed oil (3.12 g) was esterified to the more volatile FAME using dry methanol [10,11]. The ^{1}H NMR as well as GC-MS analysis were carried out on the dry seed oil FAME sample (3.00 g).

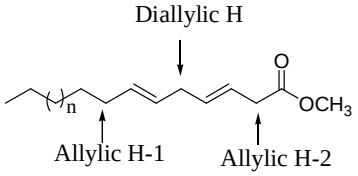
2.3 Procedure for GC-MS Analyses

Fatty acid methyl esters were analyzed using capillary column HP-5 MS (25 m × 250 µm i.d., 0.25 µm film thickness) in an Agilent 6890 GC coupled to a Waters GCT Premier mass spectrometer. The inert gas helium (99.9995%) was used as carrier gas at flow rate of 1 $mL.min^{-1}$, split injection (1 µL injection volume, split ratio, 50:1), and injection temperature of 280℃. The oven temperature was initially kept at 100℃ for 4 min then ramped at 10℃/ min to 210℃ for 4 min, then 7℃/ min to 300℃ for 3 min and held isothermally for 35 min. The MS was taken at 70 eV. The MS scan parameters included a mass range of m/z 50-600, a scan interval of 0.5 s, a scan speed of 2000 amu s^{-1}, and a detector voltage of 1.0 kV. Identification of compounds was conducted using NIST08 and WILEY8 FAME database Libraries. Mass spectrum of individual unknown compounds were compared

with the known compounds stored in the software database libraries. The name, molecular weight and structure of the components of the test materials were ascertained.

2.4 1H NMR Composition (%) of FAMEs

For a given Fatty Acid Methyl Ester (FAME), sample A, to calculate the % composition of (i). ω-3 fatty acid, (ii) di-unsaturated, (iii) mono-unsaturated and (iv) saturated fatty acids, the following Holmback equations were used (Eq. 1-4) [12].



FAME **A**

$$18{:}3_{\omega\text{-}3} = AMe._{\omega\text{-}3} / A_{Me\ (total)} \quad (1)$$

$$C_{DU} = Allyl\ 2 / A_{Me\ (total)} \times 3/2 - 18{:}3_{\omega\text{-}3} \quad (2)$$

$$C_{MU} = Allyl\ 1 / A_{Me\ (total)} \times 3/4 - 18{:}3_{\omega\text{-}3} - C_{DU} \quad (3)$$

$$C_{SAT} = 1-(1 + 2 + 3) \quad (4)$$

Where;

$A_{Me.\ \omega\text{-}3}$ = integral value for methyl protons for ω.3 fatty acid

$A_{Me\ (total)}$ = total integral value for methyl protons

Allyl 1 = integral value for allylic proton H-1

Allyl 2 = integral value for allylic proton H-2.

2.5 Antimicrobial Activity Screening

The FAMEs were screened for antimicrobial activities against *E. coli, B. subtilis, P. aeruginosa, S. aureus* and *C. albicans.* We followed methods that we recently described [13].

3. RESULTS AND DISCUSSION

The seed oil of *Baphia massaiensis* was extracted using Soxhlet extraction, concentrated in vacuo and then esterified to the corresponding fatty acids methyl esters (FAMEs) using dry methanol. The extract yield was 10.7% (3.12 g) of the cotyledon dry mass while the FAME yields was 96.1% (3.00 g) of the dried seed oil by mass. The IR, 1H NMR and GC-MS were used to characterize the *B. massaiensis* FAMEs. The IR spectrum of the FAMEs exhibited an ester carbonyl band at 1743.37 cm^{-1}, olefinic band at 3011.34 cm^{-1} and C=C stretching absorption band at 1652.84 cm^{-1}.

3.1 Characterization of FAMEs by 1H NMR

The percentage composition of the seed oil fatty acid content was estimated using 1H NMR analysis.

The key peaks were observed at 3.64 ppm assigned to ester methyl group, vinylic proton signals between 4.12 and 4.32 ppm, and allylic CH_2 signals at 2.05 and 2.77 ppm. The doubly allylic signal at 2.30 ppm were indicative of polyunsaturation (Fig. 1) [2].

The constituents of *B. massaiensis* seed oil were determined to be 20% ω-3 fatty acids, 11% mono-unsaturated, 60% di-unsaturated fatty acids and 9% saturated fatty acids. The Holmback equations [12] estimates the % composition rather than quantity of the fatty acid methyl ester. Hence, GC-MS analysis was used to determine the quantities of the fatty acid methyl esters.

3.2 Characterization of FAMEs by GC-MS Analysis

GC-MS chromatogram of the FAMEs showed seven peaks (Fig. 2) corresponding to compounds identified by comparison with those stored in the NIST database. The seven compounds identified accounted for 99.9% of the content of the FAMEs of *Baphia massaiensis*. Based on GC-MC analysis, saturated fatty acids composition was 12.2% and unsaturated fatty acids were 87.8% of the total FAMEs. Of the 87.8% of unsaturated fatty acids, 2.1% were mono-unsaturated whereas 49.7% were di-unsaturated.

The major constituents of the seed oil FAMEs were determined to be linoleic acid ($C_{18:2}$; 49.0%) and linolenic acid ($C_{18:3}$; 36.7%) methyl esters. Table 1 shows the compounds contained in the FAMEs of the seed oil of *Baphia massaiensis*.

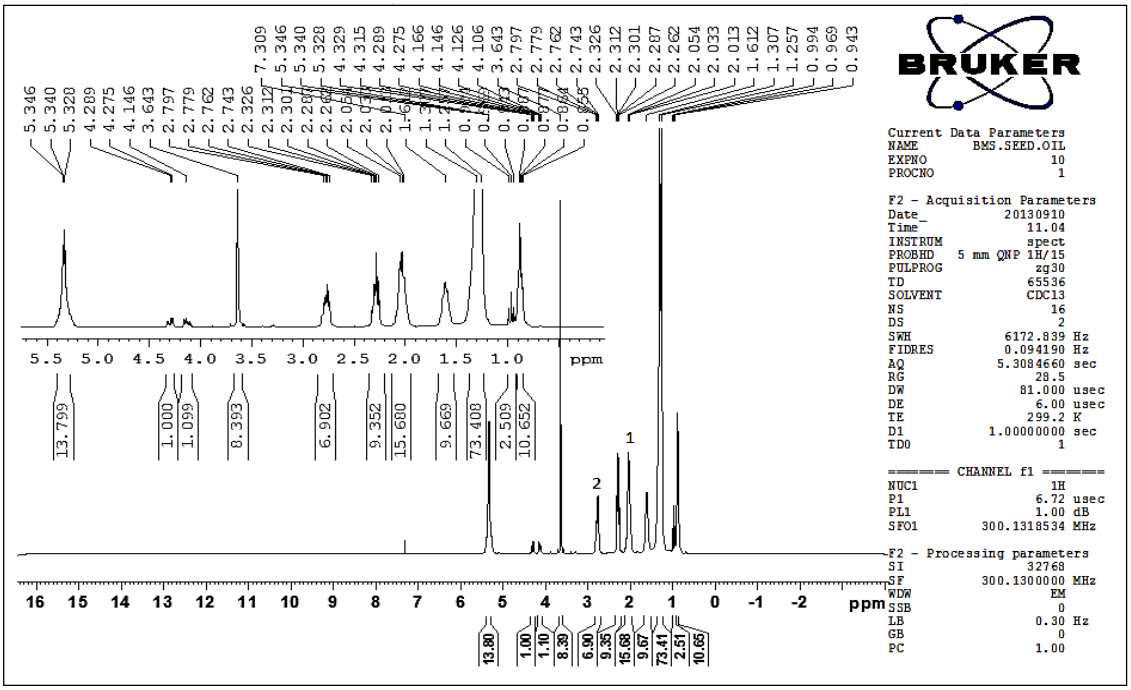


Fig. 1. 1H NMR spectrum of *B. massaiensis* FAMEs in $CHCl_3$ (where 1 = allyl H-1 and 2 = allylic H-2)

Table 1. Composition of FAMEs of *B. massaiensis* seed oil as determined by GC-MS

Peak no	Name of FAME	RT /min	Rel. %	MW	MF
1	Palmitic acid methyl ester	15.547	7.3	270.1	$C_{17}H_{34}O_2$
2	Linoleic acid methyl ester	17.917	49.0	294.1	$C_{19}H_{34}O_2$
3	Linolenic acid methyl ester	18.018	36.7	296.1	$C_{19}H_{32}O_2$
4	Stearic acid methyl ester	18.399	2.2	298.1	$C_{19}H_{38}O_2$
5	Gondoic acid methyl ester	21.940	2.1	324.2	$C_{21}H_{40}O_2$
6	Behemic acid methyl ester	25.361	2.6	354.3	$C_{23}H_{46}O_2$
7	Lignoceric acid methyl ester	27.69	≤ 0.1	382.3	$C_{25}H_{50}O_2$

These compositions of FAMEs compares well with those of edible oils. For example, Sunflower, Palm and Soya bean oils` linoleic acid composition is 46.02, 25.31 and 52.18% respectively, while their linolenic acid content is 0.12, 11.30 and 5.63%, respectively [14]. Linoleic acid is an essential fatty acid and it is a precursor to gamma linoleic acid (GLA). GLA has shown inflammation inhibiting properties [15]. A common constituent of plant oils is oleic acid, which was not detected in *B. massaiensis* seed oil. Oleic acid levels in sunflower oil normally occurs at 45.39%, while the mid-oleic sunflower has an average of 45% [14]. Oleic acid is associated with increased high-density lipoprotein (HDL) cholesterol and the hypotensive effects of olive oil (Oleic acid = 45%) [16,17]. The high level of linoleic and non-detection of oleic acid suggest that attention should be given to this variety of *B. massaiensis* seeds and to promote their enhanced production to make them available for general consumption. Fig. 2 shows the GC-MS chromatogram.

Table 2 shows that the 1H NMR method should not be used to quantify the fatty acid composition of plant seed oils because it is not accurate. Rather it can be used as a finger print in monitoring the stability of oils due to its short analysis time [12]. It may as well be used to ascertain the presence of ω-3, mono-, di and poly-unsaturated fatty acids in an oil. GC-MS on the other hand can be used to accurately determine the quantities of the individual fatty acids based on retention volumes, retention times and retention indices [5,11].

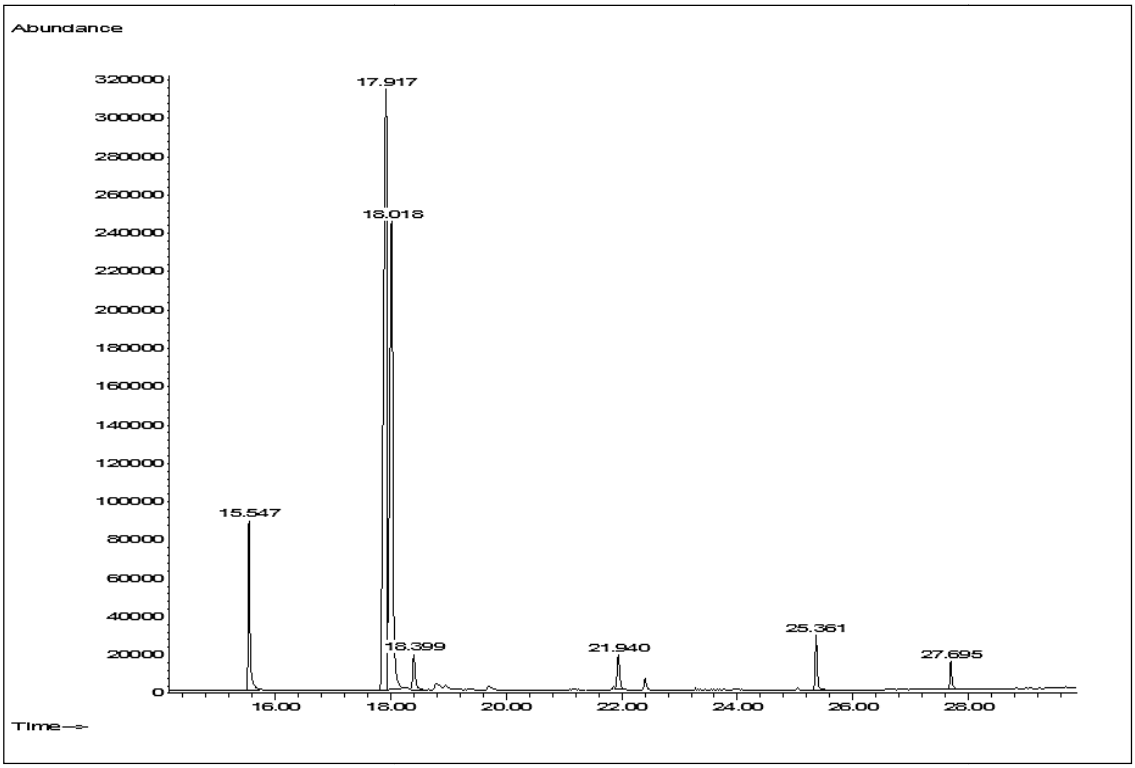


Fig. 2. GC-MS chromatogram of FAMEs of the seed oil of *B. massaiensis*

Table 2. Comparison of ^{1}H NMR and GC-MS methods

Class of FAME	% Composition of FAME in the seed oil	
	1H NMR method	GC-MS method
ω.3 fatty acid	20	36.7
Mono-unsaturated	11	2.1
Di-unsaturated	60	49.0
Saturated	9	12.2 ± 0.1

This is the first fatty acid methyl esters contents report of *Baphia massaiensis* seed oil studied by GC-MS and ^{1}H NMR. The NMR methodology indicated the presence of mono-, di and poly-unsaturated fatty acids. The GC-MS showed that linoleic acid (49.0%) and linolenic (36.7%) methyl esters were the major components of *Baphia massaiensis* FAMEs.

The FAMEs on agar well diffusion (100 µg loading) method exhibited no activities against *P. aeruginosa,* and *C. albicans,* while showing 10 mm inhibition zone against *E. coli,* and *S. aureus.* Moderate activity was shown against *B. subtilis* with a 16 mm inhibition zone. On the TLC plate agar overlay method the FAMEs were not sensitive against any organism. The good activity shown against the Gram-positive bacteria, *B. subtilis*, was similar to reported work about FAMEs from *Schotia brachypetala, Pelagonium species Quercus leucotrichophora* and *Excoecaria agallocha* [18]. The FAME extracts of these plants also had Linoleic acid (C18:2) as one of the major constituents. Linoleic acid has exhibited antibacterial activities against *S. aureus, B. subtilis* and *E. coli* [19].

4. CONCLUSION

Linoleic acid (49.0%) and linolenic (36.7%) methyl esters were the major components of *Baphia massaiensis* FAMEs. The FAMEs were active against *E. coli, S. aureus* and *B. subtilis* showing 10-16 mm inhibition zone using agar well diffusion method.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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