

# GC-MS Analysis and Antimicrobial Activities of the Non-polar Extracts of *Mundulea sericea*

O. Mazimba<sup>a,\*</sup>, I.B. Masesane<sup>a</sup>, R.R.T. Majinda<sup>a</sup> and A. Muzila<sup>b</sup>

<sup>a</sup>Department of Chemistry, University of Botswana, Private Bag 0022, Gaborone, Botswana.

<sup>b</sup>Department of Biological Sciences, University of Botswana, Private Bag 0022, Gaborone, Botswana.

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## ABSTRACT

The composition of the non-polar extracts of the leaves, stem bark and twigs of *Mundulea sericea* (Fabaceae) were analyzed using GC-MS. Eight, five and eleven components were identified from the leaves, twigs and stem bark extract, respectively. The major components were identified as: caryophyllene (43.6 %) and cadina-3,9-diene (29.7 %) in the leaf extract, amorphene (34.4 %) and valencene (17.9 %) in the stem bark extract and isodene (29.0 %) and  $\alpha$ -gurjunene (22.4 %) from the twig extract. Sesquiterpenes were the major abundant components in the non-polar extracts. The leaf, stem bark and twig extracts showed weak antibacterial and antifungal activities.

## KEYWORDS

*Mundulea sericea*, GC-MS, non-polar, sesquiterpenes, antimicrobial.

## 1. Introduction

*Mundulea* (Fabaceae; Papilionoideae) is a genus made up of 12 species.<sup>1</sup> *Mundulea sericea* (Willd.) A. Chev. is a shrub or semi-deciduous tree (2–8 m tall) and its bark, roots and seeds are used as fish poison,<sup>1-2</sup> though they are not poisonous to humans. *M. sericea* and other species in the genus have been studied phytochemically and several flavanones and flavanonols,<sup>3-6</sup> rotenoids,<sup>3,6</sup> chalcones, terpenoids and sesquiterpenoids<sup>3</sup> reported. Various biological activities have been reported for the isolates of *Mundulea*, including antibacterial and antifungal activities, cytotoxic and cancer chemopreventative activity.<sup>5-7</sup> Kalavathi<sup>2</sup> has reported minimum inhibitory concentrations of 600–1000  $\mu\text{g}/\text{mL}$  for the petroleum ether, benzene, chloroform, ethyl acetate and methanol extracts of *Mundulea sericea* stem bark against *Pseudomonas aeruginosa*, *Escherichia coli*, *Micrococcus roseus*, *Micrococcus luteus*, *Bacillus cereus*, *Bacillus megaterium*, *Staphylococcus aureus*, *Candida tropicalis* and *Aspergillus niger*.<sup>2</sup> The aim of this study was to investigate the composition of the non-polar extracts of *M. sericea* growing in the South East District of Botswana. This was done in order to provide a good profile of the non-polar extracts chemical constituents, since the phytochemical investigations on polar extracts are reported in literature.<sup>3-6</sup> The preliminary antimicrobial activities of the non-polar extracts were also determined. To the best of our knowledge, there are no previous reports about the GC-MS analysis of the non-polar extracts of *M. sericea*.

## 2. Experimental

### 2.1. Plant Material

*Mundulea sericea* was collected from Mmankgodi village in the South East District of Botswana in July 2008. A voucher specimen (82F6) has been deposited in the University of Botswana Herbarium.

### 2.2. Non-polar Extracts Preparation

The air-dried and powdered plant materials were extracted by

soaking at 25 °C for 24 h as follows: Leaves, 19.6 g powder, was extracted with *n*-hexane (500 mL) to yield 2.6 g (13.5 %) extract. Powdered stem bark (21.0 g) powder was extracted with chloroform (500 mL) and yielded 6.2 g (29.5 %) jelly extract. The dried and powdered twigs (14.0 g) were extracted with chloroform (500 mL). 1.9 g (13.2 %) black jelly extract was obtained after evaporation of the solvent with a rotary evaporator. The chloroform extracts were chromatographed on a Silica gel column eluted with *n*-hexane, to obtain the non-polar extract. The stem bark and twig extracts afforded 2.0 g (9.5 %) and 0.67 g (4.8 %) non-polar (*n*-hexane) extracts, respectively. The extract dissolved in chloroform were individually dried over  $\text{Na}_2\text{SO}_4$  and stored at 4 °C prior to qualitative analysis. The *n*-hexane extracts yields (%) were based on the dried weight of plant samples.

### 2.3. GC-MS Analysis

GC-MS analyses were performed using a capillary column HP-5 MS (25 m  $\times$  250  $\mu\text{m}$  i.d., 0.25  $\mu\text{m}$  film thickness) in an Agilent 6890 gas chromatograph coupled to a Waters GCT Premier mass spectrometer. The carrier gas was helium with a constant flow rate of 1 mL  $\text{min}^{-1}$ . The oven temperature was initially kept at 50 °C for 6 min then ramped at 4 °C  $\text{min}^{-1}$  to 300 °C and held isothermally for 30 min. Solutions of the samples (100 ppm in chloroform) were injected manually at 250 °C. Injection volume was 1.0  $\mu\text{L}$  in the splitless mode. Mass spectra were obtained by EI at electron energy of 70 eV.

### 2.4. Identification and Quantification of Constituents

The relative percent composition of the non-polar extract constituents were determined by computerized peak area measurements using the internal normalization method. Identification of components was conducted, based on GC retention times, on an HP-5MS capillary column, and by computer matching of the acquired mass spectra with the NIST 05 L Mass Spectral Library.<sup>8,9</sup> Retention indices were also compared to literature values. An agreement above 95 %, of the spectra, was considered for identification of constituents.

\*To whom correspondence should be addressed. E-mail: mazimbaof@yahoo.com

### 2.5. Antimicrobial Testing

The antimicrobial activities of extracts and compounds were evaluated using agar-overlay bioautography method.<sup>10</sup> The microorganisms used were: *Staphylococcus aureus* (ATCC 9144), *Escherichia coli* (ATCC 11229), *Bacillus subtilis* (ATCC 6633), *Pseudomonas aeruginosa* (NCTC 10332) and *Candida albicans* (ATCC 10231). 24-hour-old (37 °C) cultures were used. The microorganisms were obtained from the Department of Biological Sciences, University of Botswana. Chloramphenicol and miconazole were used as standards for bacteria and fungi, respectively. Stock solutions of 10 mg mL<sup>-1</sup> (MeOH and CHCl<sub>3</sub>) of the test sample were prepared and serially-diluted to obtain concentrations of 5.0, 1.0, 0.05, 0.001 mg mL<sup>-1</sup>. An aliquot of 10 µL of each concentration was spotted on Merck pre-coated silica gel 60 HF<sub>254</sub> TLC plates (0.25 mm thickness; 10 cm × 10 cm), corresponding to loading quantities of 100, 50, 10, 0.5 and 0.01 µg. The spots were of the same size, and the solvent was allowed to evaporate in the fume hood. The seed layers were prepared by inoculating 10 mL aliquot of culture into 100 mL agar (Oxoid: Sabouraud Dextrose) solution. Using a sterile Pasteur pipette the TLC plates were overlaid with the agar. Plates were run in triplicates. After the medium congealed the TLC plates were incubated at 37 °C (*Staphylococcus aureus*, *Escherichia coli*) and 25 °C (*Bacillus subtilis*, *Pseudomonas aeruginosa*, *Candida albicans*) for 24 h. The bioautograms were sprayed with an aqueous solution of thiazoyl blue (methylthiazoyltetrazolium bromide; 200 mg in 100 mL distilled water) and further incubated for 4 hours after which results were scored. White spots against purple background indicated inhibition zones. The minimum inhibitory quantity (MIQ in µg) was taken as the lowest loading quantity to exhibit an inhibition zone.

### 3. Results and Discussion

The chemical profiles of the analyzed non-polar extracts and the percentage composition of the individual constituents are given in Table 1. Twenty-two components tentatively identified in the *n*-hexane extract of *M. sericea*, accounted for 99.40 %, 99.50 % and 97.70 % of the identified components in the leaves, stem bark and twigs extracts. The main constituents from the leaves were caryophyllene (43.60 %) and cadina-3,9-diene (29.70 %), while in the stem bark they were  $\alpha$ -amorphene (34.40 %) and valencene (17.90 %), and the twigs abundant constituents were isodene (29.00 %) and  $\alpha$ -gurjunene (22.40 %).

The phytochemical work, which has been done on the polar extracts of *Mundulea*, has not produced any of the metabolites reported here.<sup>3-6</sup> The only sesquiterpene reported before from the genus *Mundulea* is 8 $\alpha$ -acetoxyelemol from the stem bark of *Mundulea chapelieri*.<sup>6</sup> Copaene was the common component between the leaves and stem bark, while spathulenol was common between the twigs and stem bark. The other components of the leaf, stem bark, and twigs were different, which is a common occurrence within the compositions of different plant

**Table 1** GC-MS analysis of *Mundulea sericea* non-polar extract.

Components	R.I. <sup>a</sup>	R.I. <sup>b</sup>	% composition <sup>c</sup>
<b>Leaves (L)</b>			
Copaene	1378	1379 <sup>14</sup>	4.38
Caryophyllene	1405	1413 <sup>12</sup>	43.60
<i>cis</i> -Bisabolene	1413	1420 <sup>12</sup>	0.85
Humulene	1452	1454 <sup>15</sup>	8.41
Cadina-3,9-diene	1514	1517 <sup>16</sup>	29.70
Humulane-1,6-dien-3-ol	1544	1596 <sup>15</sup>	4.04
Caryophyllene oxide	1582	1586 <sup>16</sup>	7.00
A	1704	1715 <sup>11</sup>	1.20
<b>Twigs (T)</b>			
$\alpha$ -Gurjunene	1476	1472 <sup>14</sup>	22.41
Isodene	1494	1500 <sup>12</sup>	29.03
Eudesma-3,7(11)-diene	1508	1510 <sup>14</sup>	11.31
$\alpha$ -Guaiane	1542	1545 <sup>12</sup>	16.76
Spathulenol	1578	1581 <sup>16</sup>	18.21
<b>Stem bark (SB)</b>			
Copaene	1378	1379 <sup>14</sup>	10.62
$\alpha$ -Elemene	1392	1393 <sup>15</sup>	5.47
$\alpha$ -Iraldene	1450	1452 <sup>15</sup>	2.20
Valencene	1464	1465 <sup>15</sup>	17.90
$\alpha$ -Amorphene	1495	1494 <sup>15</sup>	34.37
B	1509	1510 <sup>11</sup>	7.44
C	1512	1515 <sup>11</sup>	10.84
$\alpha$ -Santalol	1556	1558 <sup>16</sup>	1.50
Spathulenol	1578	1581 <sup>14</sup>	18.21
D	1583	1596 <sup>14</sup>	3.20
Larixol	1673	1680 <sup>12</sup>	0.60
Sesquiterpene hydrocarbons	L		87.20
	T		79.50
	SB		68.40
Oxygenated sesquiterpenes	L		12.20
	T		31.10
	SB		18.20
Total identified	L		99.40
	T		97.70
	SB		99.50

<sup>a</sup> Retention indices relative to C<sub>9</sub>–C<sub>24</sub> n-alkanes on the HP 5MS column.

<sup>b</sup> Retention indices according to the literature.

<sup>c</sup> % composition based on peak areas calculated in GC on HP 5MS column.

A: 6,10,14-trimethyl-2-pentadecanone, B: (6E)-3,7,11-trimethyl-1,6,10-dodecatrien-3-ol, C: 1,5,5,8-tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene, D: isoaromadendrene epoxide.

parts.<sup>11</sup> Common components of plant essential oils and non-polar extracts such as caryophyllene, bisabolene, elemene and an oxygen containing sesquiterpene, spathulenol<sup>9,12,13</sup> were found in *M. sericea* non-polar extracts. Sesquiterpenes were found to be the main components. The amount of sesquiterpene hydrocarbons was 87.20 %, 68.40 % and 79.50 % in the leaf, stem bark, and twig extracts. The composition of the oxygenated sesquiterpenes was 12.20, 31.10 and 18.20 % for the non-polar extract from the leaves, stem bark, and twigs of *M. sericea*.

Table 2 shows the antimicrobial activities of the non-polar extracts of *M. sericea*. The stem bark extract showed activities

**Table 2** Antimicrobial activity of *Mundulea sericea* non-polar extract.

Extract	Microorganism and MIC in µg				
	<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>
Twigs	50	10	10	10	10
Stem bark	10	0.5	10	10	0.5
Leaves	50	100	100	100	na

Chloramphenicol 0.001 µg, Miconazole 0.001 µg.

against *B. subtilis* and *C. albicans* (MIQ = 0.5 µg). The antibacterial activity exhibited by the non-polar extracts against Gram-negative bacteria conforms to literature, which reports low sensitivities against Gram-negative bacteria.<sup>17,18</sup> Based on the literature data<sup>3–6</sup> about the biological activities of *Mundulea*, it should be noted that besides the reported activities of the volatile compounds, there is a distinct possibility that the non-volatile constituents are also contributing to the bioactivity of the plant, however minimal.

The leaves show low antifungal activity even though their main component, caryophyllene (43.60 %) was reported to have exhibited mycelial growth inhibition against *F. oxysporum*<sup>19</sup>. The low potencies of the non-polar extracts were *Mundulea sericea* (Willd.) *Mundulea sericea* (Willd.) found to be in good agreement with the literature.<sup>2</sup> Flavanoids, quinones and coumarins had been preliminarily tested and reported from the petroleum ether extract, this non-polar extract was reported to have shown minimum inhibitory concentrations greater than 100 µg mL<sup>-1</sup> against *P. aeruginosa*, *E. coli*, and *S. aureus*.<sup>2</sup>

#### 4. Conclusions

Twenty-two components were identified from the non-polar (*n*-hexane) extracts of *M. sericea* leaves, stem bark and twigs. Sesquiterpenes were identified as the major components, accounting for 93.50 %, 97.70 % and 97.90 % of stem bark, twigs and leaves extracts, respectively. The non-volatile extracts' antibacterial and antifungal activities have previously been reported,<sup>2–5</sup> but here the antimicrobial screening indicated weak antimicrobial activities of the non-polar extracts against *S. aureus*, *B. subtilis*, *E. coli*, *P. aeruginosa* and *C. albicans*.

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