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Extraction, chemical composition, and antioxidant activity analysis of essential oil from *Schinus molle* medicinal plant found in Botswana

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Abstract
The aim of this study was to investigate the effects of hydrodistillation (HD) and ultrasonic-assisted hydrodistillation (UAHD) on the yield, chemical composition and antioxidant activity of the essential oils extracted from *Schinus molle* plants cultivated in Botswana. Hydrodistillation with a Clevenger apparatus and ultrasonic-assisted hydrodistillation at 300 watts (W) and 50 watts (W) were used as the extraction methods of choice to extract the essential oil from the plant leaves. The average percentage yield of essential oil obtained by HD was 1.91 ±0.27%, while that obtained by UAHD at 300 W was 2.33 ± 0.21%and UAHD at 50 W was 1.14 ± 0.11%. Major compounds found in the essential oil extracted by HD include limonene (13.93%), elemol (12.35%), α-phellandrene (10.82%), aromadendrene (9.11%), 6-epi-shyobunol (6.75%), α-eudesmol (5.14%), β- cadinene (4.61%), and α-muurolene (4.29%). Major compounds identified in the essential oil extracted by UAHD (at 300 W and 50 W) were elemol (15.23%, 24.04%), 6-epi-shyobunol (12.39%, 12.32%), aromadendrene (9.92%, 9.81%), and α-eudesmol (9.74%, 8.37%). The antioxidant activity test was performed using 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. The IC_{50} of essential oil obtained by HD was 134.3 µg/ml, while the essential oil extracted by UAHD at 300 W had IC_{50} of 102.3 µg/ml, and that of UAHD at 50 W was 226.0 µg/ml. It is worth noting that this is the first time that this study was done for this plant species cultivated in Botswana.

Keywords *S. molle*; chemical composition; hydrodistillation; ultrasonic-assisted hydrodistillation; antioxidant activity

1. Introduction
*Schinus moll*e, known as California pepper tree belongs to the "*Anacardiaceae*" family [1]. This medicinal plant is indigenous in Central America and South America, whereas in Botswana, it is an exotic plant usually cultivated by communities in their backyards [2, 3]. Studies have reported *S. molle* as sources of herbal medicine in traditional communities [2, 4]. In South Africa, *S. molle* leaves are used to treat influenza, depression, hypertension, and arrhythmias [1]. In Botswana, it is used as a treatment for flu and as an ornamental tree. The treatment involves boiling the plant leaves and then subjecting the patient to the steam for inhalation.

Essential oils extracted from *S. molle* leaves have been shown to be rich in biologically active secondary metabolites which justifies the ethnomedicinal use [2, 4, 5]. Previous studies on *S. molle* essential oil shows the following as major biological compounds: bicyclogermacrene, β-caryophyllene, spathulenol, germacrene-D, caryophyllene oxide, terpene-4-ol, α-pinene, sabinene, limonene, d-limonene, ß-pinene, cubenol, verbenone, 1,8-cineole, epi-α-cardinal, γ-cadinene, α-eudesmol, 7-epi-α-eudesmol, 1,10-di-epi-cubenol, α-phellandrene, and β-phellandrene [2–4, 6–10]. Furthermore, studies have shown that *S. molle* essential oils have significant antioxidant activity [9]. For example, some studies reported antioxidant activity with IC_{50} of 47.4 µg/ml [2], 3586.9 mg/l [10], 36.3 mg/ml [11], and 100.0 µg/ml [4]. The antioxidant and medicinal properties of essential oils depend much on its chemical composition [12].

Environmental factors such as temperature, light, altitude, slope, precipitation, water status, and salinity have an influence on the biosynthesis of essential oils and secondary metabolites in plants [5, 10, 13]. These factors can also affect the yield of the essential oils. Studies have reported the percentage yield of *S. molle* essential oil obtained by hydrodistillation to range from 1.09% to 2.0% depending on where they were extracted [2, 4, 7–9, 11, 14]. Extraction methods

have also been found to greatly influence the chemical composition and yield of essential oils. Consequently, modern techniques such as ultrasonic assisted hydrodistillation have been explored to improve the efficiency of the classical extraction methods. Interestingly, a survey of recent literature does not show the investigation on effects of UAHD on the yield and chemical composition of essential oils from *S. molle*. Furthermore, studies have not also been done on the yield, chemical composition and antioxidant capacity of essential oils extracted from *S. molle* plants cultivated in Botswana. Therefore, the primary objective of this study was to investigate the effects of HD and UAHD on the yield, chemical composition and antioxidant activity of the essential oils extracted from *S. molle* plants grown in Botswana.

2. Materials and methods

2.1 Plant materials

This study was carried out in the Chemical and Forensic Sciences Department at Botswana International University of Science and Technology (BIUST) in Palapye-Botswana, between May 2019 and November 2020. The leaves of *S. molle* were harvested at Lotsane area (22°33'S 27°08'E) in Palapye village. The harvesting time was between 8 am, and 9 am. Dr Rantong Gaolatlhe, a botanist from the Biology & Biotechnological Sciences Department at BIUST, verified the plant name.

2.2 Preparation of plant materials for the extraction

Plant leaves were dried for seven days in the research laboratory at room temperature (24°C-27°C) according to Abir *et al*. (2016) [10] to achieve better yield. Leaves were crushed into powder using a blender (brand name.: Russell Hobbs, Model No.: 14449, 240 V, 400 W).

2.3 Hydrodistillation (HD)

The crushed leaves (50 g) were put into a 2000 ml two-necked conical flask with a magnetic stirrer to achieve homogeneity. A thermometer was connected through one of the two necks to monitor the temperature of the biomass during the distillation process. Distilled water (1200 ml) was added to the flask and the mixture was stirred and distillation was done with Clevenger apparatus for 3 hours after collecting the first drop. The essential oil was extracted from the distillate with pentane and dried on anhydrous sodium sulphate. The solvent was evaporated on a water bath maintained at 40°C to yield a clear yellow essential oil. This extraction process was performed in triplicates, and the percentage yield of the essential oil were calculated using the following formula (Eq. 1):

$$\% \text{ yield} = \frac{\textit{Mass of essential oil obtained}}{\textit{Mass of the crushed leaves used}} \times 100\% \quad (1)$$

A one-way ANOVA (statistical analysis) with Microsoft excel was used to determine statistical differences among percentage yield mean values. The results from ANOVA analysis were significantly different hence Tukey Kramer Multiple Comparison was used to perform Post Hoc Test [15].

2.4 Ultrasonic Assisted Hydrodistillation (UAHD)

Warm distilled water (1200 ml, 70°C) was added to the sample (50 g) in a two-necked conical flask and then soaked for 20 minutes. After soaking, the sample was subjected to the ultrasound (12.0 L ultrasonic water bath with timer function, model.: 704, brand name.: LABOTEC Sonic Clean) for 25 minutes. The ultrasonic water bath temperature was maintained at 70°C, and the ultrasonic power was set at 50 W (low) and 300 W (high). After sonication, the plant material was subjected to hydrodistillation for 3 hours in a Clevenger apparatus. The essential oil was extracted from the distillate with pentane and dried on anhydrous sodium sulphate. The solvent was evaporated on a water bath maintained at 40°C to yield a clear yellow essential oil. This extraction process was performed in triplicates, and the percentage yield of the essential oil were calculated using the formula in Eq. 1. Statistical analysis of the percentage yield was done as outlined in the above section.

2.5 Analysis of essential oils by GC-FID and GC-MS

GC-FID: The instrument used to analyze *S. molle* essential oils was the Agilent gas chromatography coupled with FID (GC-7890B). The capillary column used was HP-5MS (Hewlett Packard, CA, USA). The capillary column was 30 m × 320 µm × 0.25 µm (bonded with 95% dimethyl silicone as a stationary phase) and coated with 5% phenylmethyl silicone (0.25 mm phase thickness). The oven temperature was programmed to 70°C for 5 minutes, rising to 300°C at 5°C/min. The injector temperature and detector temperature were 250°C, and the carrier gas was helium at 80 kPa with a flow rate of 0.5 ml/m. The injection sample volume was 0.2 µl and the injection mode was split, with a split ratio of 1:10.

GC-MS: The Agilent 7890B GC system, coupled with an Agilent 5977A mass spectrometer detector (MSD) with electron impact ionization (70 eV), was employed to analyze the essential oil. The capillary column used was HP-5MS (Hewlett-Packard, CA, USA) (30 m × 320 µm × 0.25 µm), coated with 5% phenylmethyl silicone, 95% dimethyl silicone, 0.25 mm film thickness. The injection volume was 0.2 µl, the temperature of the oven was programmed to rise from 70°C to 300°C with a 5°C/min rate, and the carrier gas was helium with a 0.5 ml/min flow rate; the injection mode was split, with a split ratio of 10:1. The pressure and average velocity of the carrier gas were 3.1561 psi and 10.769 cm/s, respectively. Lastly, scan time was 1s, and the mass range was between 40-300 m/z.

Retention indices were calculated based on the analysis of a homologous series of n-alkanes standard from C_7 to C_{30} on similar temperature-programmed conditions as the essential oil sample. Further confirmation of chemical compounds involved checking the MS library search (NIST and Willey) containing the known compounds, NIST Chemistry WebBook (SRD 69), PHEROBASE library, and the literature published data [10, 16–20]. The relative percentage composition of each compound was calculated on the basis of their peak areas without correction for response factors.

2.6 Antioxidant test using the DPPH free radical assay

The DPPH (1, 1-diphenyl-2-picrylhydrazyl) assay preparations and procedure (volumes of the sample and DPPH) were followed according to Simionatto *et al*. (2011) [4] with modifications. The DPPH methanolic solution was prepared at a concentration of 0.004% (40 µg/ml) by dissolving 10 mg of DPPH into 250 ml of methanol. The essential oil was then prepared in different concentrations (30µg/ml, 100µg/ml, and 200µg/ml) in methanol, of which 200 µl of each essential oil concentration was added to 2 ml of a 0.004% methanolic solution of DPPH in separate test tubes. The mixture was shaken vigorously to achieve homogeneity and placed in a dark place (oven) for 30 minutes

at room temperature (24°C-27°C).
The absorbance was measured against the blank (methanol) using the Ultraviolet-Visible Spectrophotometer (Model: Evolution 201, Serial Number: 5A4P045003) at 517 nm wavelength. This procedure was conducted in triplicates, and the mean value was recorded. The inhibition percentage (I%) of DPPH scavenging activity was calculated using the %Inhibition equation below (Eq. 2):

$$\% \text{ Inhibition} = \left[\frac{(Absorbance\ of\ the\ Control - Absorbance\ of\ the\ Sample)}{Absorbance\ of\ the\ Control}\right] \times 100\% \qquad (2)$$

The *absorbance of the control* was the absorbance of methanolic DPPH only, and the *absorbance of the sample* was the absorbance of essential oil and DPPH mixture. Ascorbic acid was tested following the same procedure and used as a positive control.
One-way ANOVA (statistical analysis) with Microsoft excel was used to determine the statistical differences on the results. Kramer Multiple Comparison was used to perform Post Hoc Test to separate the mean values.

3. Results and discussion

3.1 Percentage yields of *S. molle* essential oil by different extraction methods

The percentage yields for *S. molle* essential oils obtained by HD, UAHD at 50 W, and UAHD at 300 W were calculated and presented in Table 1. The statistical analysis of the results showed that the mean value of HD to UAHD at 50 W and UAHD at 50 W to UAHD at 300 W were significantly different, whereas HD to UAHD at 300 W were not significantly different.
As presented in Table 1, the yield of the essential oil extracted by HD is 1.91%. This recovery yield is higher than most yields reported in the literature. For example, Simionatto *et al*. (2013) and Duarte *et al*. (2018) on their *S. molle* essential oil studies reported the yield of 1.25% [4] and 0.36% [7], respectively. Diaz *et al*. (2008) [11] reported a recovery yield of 1.2%, which is still much lower than the essential oil extracted by HD in this study. On the other hand, the percentage yield of the essential oil obtained by HD in this study is comparable to other studies reported in the literature. For example, similar recovery yields of 1.86% [10], 1.88% [9], 2.0% [8], and 2.1% [14] have been reported.

Table 1: Percentage yield of *S. molle* essential oil by different extraction methods

Extraction method	%Yield (w/w relative to dry material weight)
HD	1.91 ± 0.27%
UAHD at 300 W	2.33 ± 0.21%
UAHD at 50 W	1.14 ± 0.11%

* $P = 0.001$, $P < 0.05$, One-way ANOVA analysis with Microsoft excel

The percentage yield of the essential oil extracted by UAHD at 300 W is 2.33% (Table 1). This recovery yield is higher than the reported yields obtained through HD in literature [14]. Table 1 also shows that UAHD at 300 W has a higher percentage yield than the HD in this study (1.91%). The ultrasound with low frequency (between 20 kHz-50 kHz) helps to burst the oil sacs, thus bursting cell walls and cell membranes of essential oil reservoirs. The breaking of essential oil reservoirs allows the plant's cell content to freely mix up with the solvent (water), thereby increasing the efficiency in extracting the extractable compounds with an increased yield [21]. Li *et al*. (2004) made similar observations with an increase in oil yield after ultrasonic pretreatment compared to the non-sonicated soybean samples (extracted by hexane and isopropanol) [22].
The average percentage yield for essential oil extracted by UAHD at 50 W is 1.14% (Table 1). This yield is much lower than that obtained by extracting with UAHD at 300 W and HD. The relationship between output power and frequency produced may best justify this observation. The output power of the ultrasonic bath is inversely related to the amount of frequency made. The higher the power, the lower the frequency, and the lower the power, the higher the frequency [23]. Reducing the ultrasonic power to 50 W increases the frequency, hence reducing the cavitation intensity, which reduces the essential oil yield recovery efficiency [24]. The cavitation bubbles had a short time to grow and collapse under the ultrasonic oscillations in UAHD at 50 W. As such, the collapsing of the bubbles had low pressure to cause rapture of the plant cells. Altemimi *et al.* (2015) reported similar observations on the effects of ultrasonic treatments on the extraction of spinach extracts at 37 kHz and 80 kHz ultrasonic frequency; 37 kHz (low frequency) was more effective by giving a higher yield than 80 kHz (high frequency) [25].

3.2. Chemical composition of the *S. molle* essential oils

3.2.1 Chemical composition of the *S. molle* essential oil extracted by HD

The essential oil extracted by HD (Fig. 1) has seventy-five compounds. Seventy-two of the compounds were identified, forming 99.83% of the essential oil. Sesquiterpenes makes up 65.25% of the essential oil, and 33.27% of the essential oil is due to the monoterpenes.
The most abundant compounds in this essential oil are limonene (13.93%), elemol (12.35%), α-phellandrene (10.82%), and aromadendrene (9.11%). Other compounds with relatively high abundances in this essential oil are 6-epi-shyobunol (6.75%), α-eudesmol (5.14%), β- cadinene (4.61%), and α-muurolene (4.29%) (Fig. 2, Table 2).

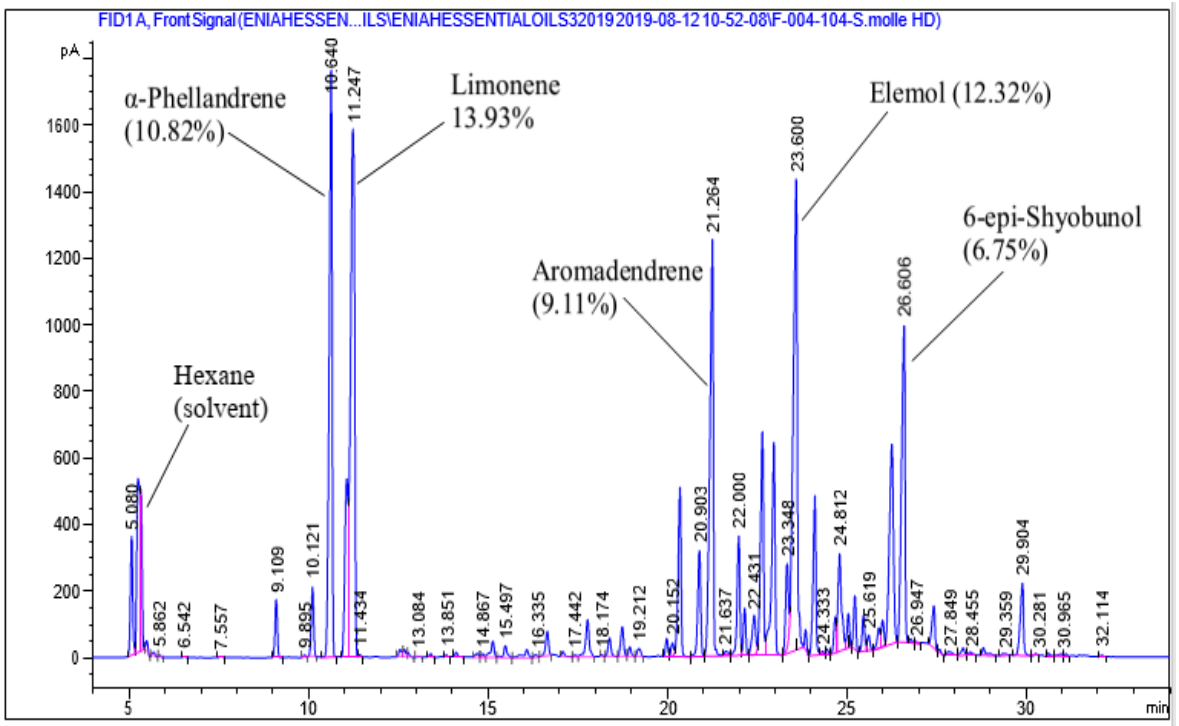

Fig 1: GC-FID chromatogram of *S. molle* essential oil extracted by HD

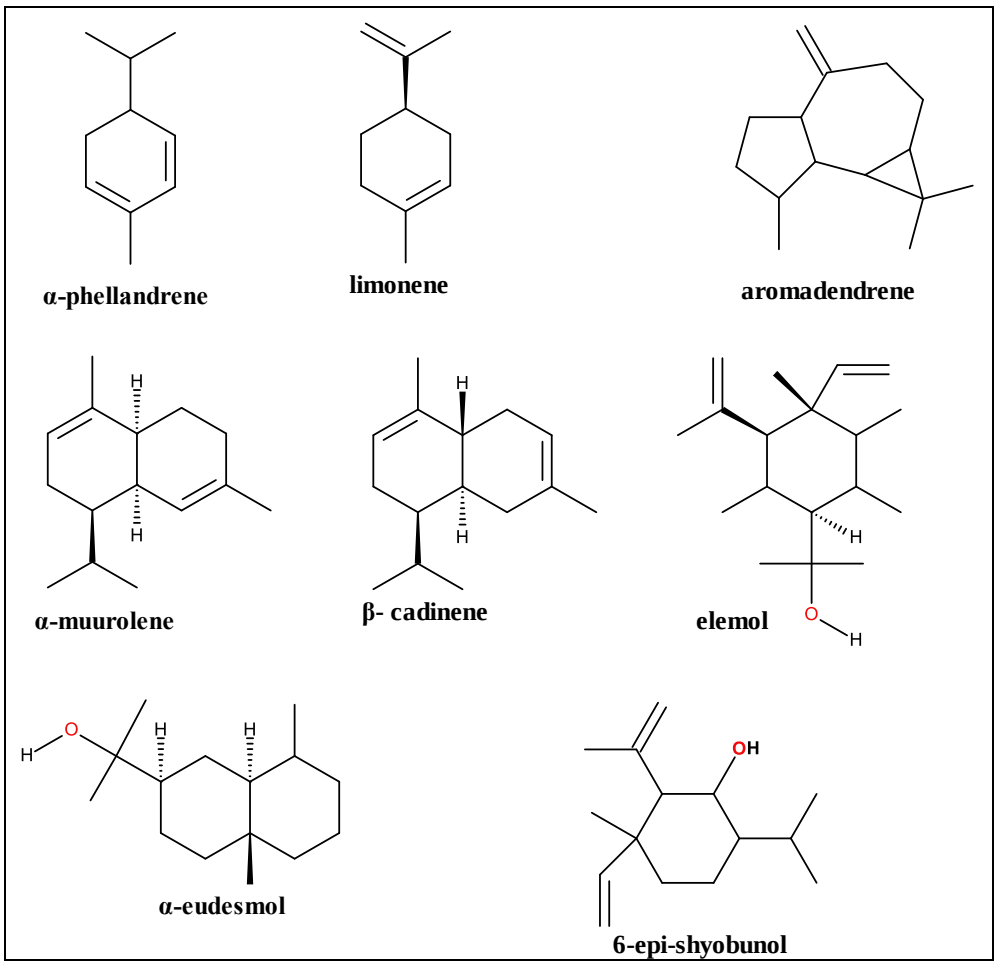

Fig 2: Chemical structure of the major compounds

3.2.2. Chemical composition of the essential oil extracted by UAHD at 300 W

The essential oil extracted by UAHD at 300 W has seventy-one compounds. This study identified sixty-seven compounds that constitute 99.77% of this essential oil. The sesquiterpenes makes up 85.71% of the essential oil while 11.5% of the essential oil components are monoterpenes. Fig. 3 below is the chromatogram from the GC-FID for the essential oil extracted by UAHD at 300 W.

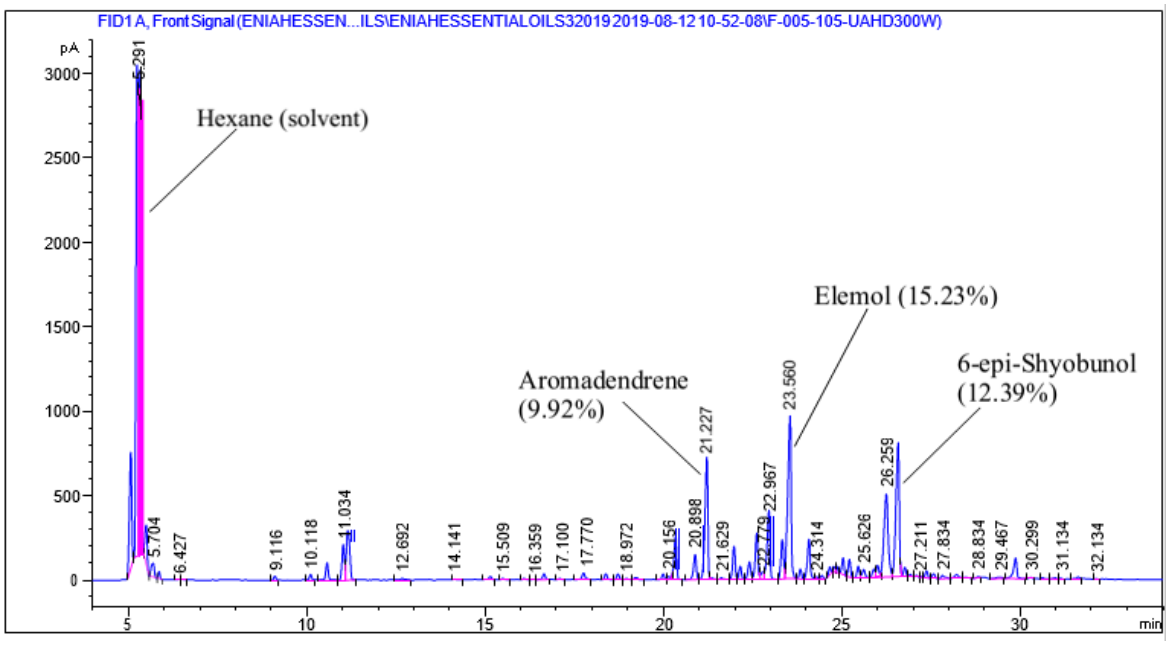

Fig 3: GC-FID chromatogram of *S. molle* essential oil extracted by UAHD at 300 W

The most abundant compounds in this essential oil are elemol (15.23%), 6-epi-shyobunol (12.39%), aromadendrene (9.92%), and α-eudesmol (9.74%). The complete profile of the components of this essential oil is presented in Table 2. The total number of compounds in this essential oil is four compounds less than in the essential oil extracted by HD. According to Li *et al*. (2004), some extractable compounds may have been deteriorated/oxidized upon being exposed to UAHD at 300 W treatment. Their results show that the essential oils subjected to ultrasonication had less unsaturated compounds compared to the sample that has been extracted by HD [22]. The relative percentage abundance of major compounds in this essential oil is higher than those identified in HD essential oil. Such compounds with higher concentration may have also contributed to the higher recovery yield observed in UAHD at 300 W than HD.

3.2.3. Chemical composition of the essential oil extracted by UAHD at 50 W

The essential oil extracted by UAHD at 50 W has twenty-three compounds. All the compounds in this essential oil were identified. Of the identified compounds, 97.96% are sesquiterpenes and 2.04% are monoterpenes. Below is Fig. 4, showing the chromatogram of the essential oil.

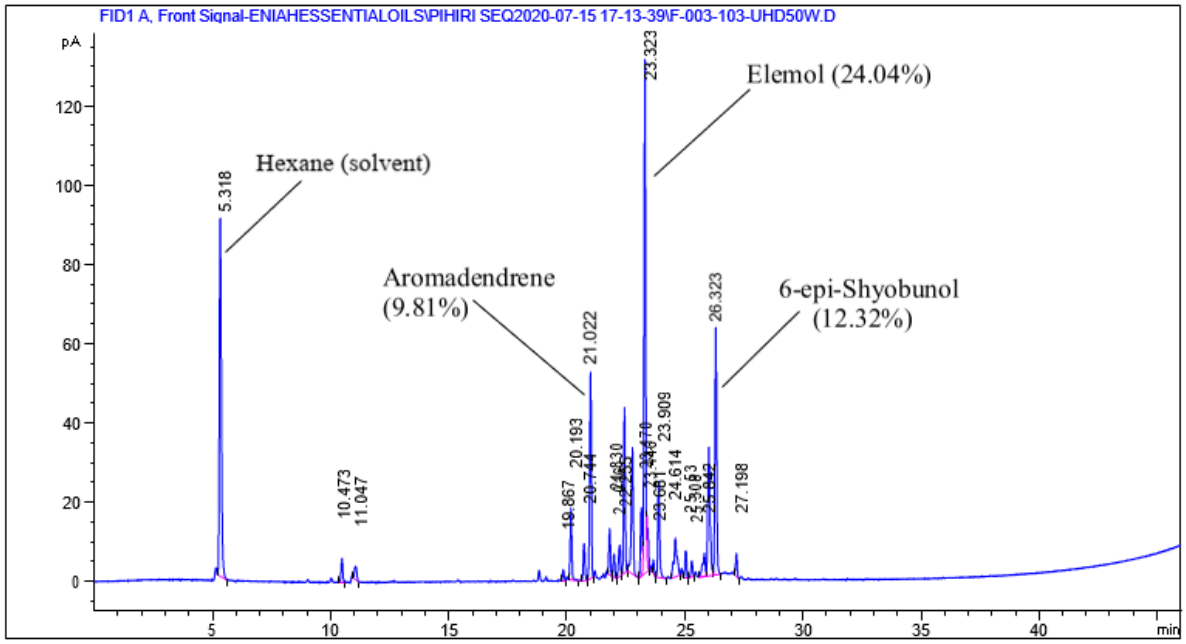

Fig 4: GC-FID chromatogram of *S. molle* essential oil extracted by UAHD at 50 W

The most abundant compounds identified in this essential oil are elemol (24.04%), 6-epi-shyobunol (12.32%), aromadendrene (9.81%), and α-eudesmol (8.37%) (Fig.4). Table 2 gives a complete profile of the essential oil components. The total number of compounds in this essential oil are few compared to the essential oil extracted by both HD and UAHD at 300 W. Some compounds are not present in the essential oil extracted by UAHD at 50 W, which further supports the conclusion that some sensitive compounds may have been oxidized or destroyed upon being subjected to the

ultrasonic treatment at 50 W. Limonene is present in UAHD at 300 W with only 4.78%, and upon being treated with UAHD at 50 W, it is completely absent in the essential oil. However, in HD limonene is present in 13.92% relative abundance.

Table 2: RI and concentration of chemical compounds identified in *S. molle* essential oil extracted by HD and UAHD (at 50 W and 300 W)

Chemical compounds	Kováts RI	HD Conc.%	UAHD at 50 W Conc.%	UAHD at 300 W Conc.%
1-Pentanol	759	-	-	0.05± 0.01
2-Pentenol, (Z)	772	0.01± 0.01	-	0.05± 0.00
3-Hexen-1-ol, (E)	851	0.02± 0.01	-	-
α-Pinene	941	0.86± 0.01	-	0.25± 0.00
β-Pinene	980	0.03± 0.01	-	-
β-Myrcene	991	1.07± 0.02	-	0.35± 0.03
α-Phellandrene	1008	10.82 + 0.3	1.20± 0.02	1.24± 0.02
p-Cymene	1033	3.64± 0.01	0.84± 0.01	2.48± 0.04
Limonene	1037	13.93± 0.05	-	4.78± 0.01
β-Phellandrene	1048	0.07± 0	-	-
α-Pinene oxide	1095	0.13± 0.01	-	-
Linalool	1100	0.14±0.02	-	0.21± 0.00
Nonanal	1104	0.07± 0.01	-	-
trans-para-2,8-menthadien-1-ol	1116	0.02± 0.03	-	-
cis-p-mentha-2,8-dien-1-ol	1129	0.05± 0.01	-	-
Camphor	1146	0.03± 0.02	-	-
Borneol	1158	0.07± 0.02	-	0.07± 0.03
1,8-menthadien-4-ol	1180	0.06± 0.02	-	-
Cryptone	1186	0.04± 0.02	-	-
Isopiperitenol	1197	0.32± 0.03	-	0.35± 0.02
Trans- (+)-Carveol	1211	0.21± 0.01	-	0.14± 0.01
cis-p-mentha-1(7),8-dien-2-ol	1234	0.20± 0.01	-	0.11± 0.00
Carvacrol, methyl ether	1244	0.03± 0.05	-	0.05± 0.00
Geraniol	1256	0.43± 0.01	-	0.53± 0.01
Phellandral	1273	0.09± 0.01	-	0.11± 0.02
Bornyl acetate	1286	0.02± 0	-	-
Thymol	1299	0.86± 0	-	0.71± 0.06
Carvacrol	1316	0.02± 0.03	-	-
Methyl decanoate	1325	0.32± 0.01	-	0.53± 0.02
Elemene	1339	0.54± 0.01	-	0.53± 0.01
α-Terpinyl acetate	1348	0.18± 0.03	-	0.12± 0.00
Eugenol	1358	0.18± 0.01	-	0.25± 0.01
β-Elemene	1391	0.32± 0.01	0.48± 0.01	0.35± 0.01
Isoitalicene	1397	0.20± 0.01	-	0.21± 0.01
α-Gurjunene	1406	3.21± 0.02	3.35± 0.01	3.36± 0.03
β-Caryophyllene	1422	2.04± 0.01	1.79± 0.00	1.95± 0.01
Aromadendrene	1442	9.11± 0.03	9.81± 0.02	9.92± 0.03
Alloaromadendrene	1459	0.12± 0.01	-	0.14± 0.02
α-Amorphene	1475	2.04± 0.01	2.03± 0.01	2.30± 0.02
Germacrene-D	1482	0.64± 0.01	1.08± 0.02	0.89± 0.03
Valencene	1493	0.86± 0.1	1.79± 0.01	1.42± 0.02
α-Muurolene	1501	4.29± 0.02	7.42± 0.02	3.54± 0.03
γ-Cadinene	1508	-	-	0.53± 0.02
β-Cadinene	1516	4.61± 0.02	7.30± 0.01	5.84± 0.03
α-Copaen-11-ol	1533	1.39± 0.03	2.99± 0.04	3.01± 0.03
α-Cadinene	1537	-	1.56± 0.03	-
Elemol	1544	12.32± 0.03	24.04± 0.54	15.23± 0.02
Germacrene B	1555	0.21± 0.05	0.60± 0.03	0.71± 0.02
Palustrol	1567	2.89± 0.11	4.90± 0.04	3.19± 0.01
Spathulenol	1576	0.04± 0.01	-	0.11± 0.01
Caryophyllene oxide	1582	0.10± 0.01	-	0.21± 0.01
Viridiflorol	1589	0.54± 0.02	3.35± 0.20	0.35± 0.01
Ledol	1596	-	-	0.32± 0.02
α-Ylangene	1600	2.25± 0.12	-	0.35± 0.01
Oplopenone	1608	0.54± 0.03	1.32± 0. 20	1.06± 0.02
10-epi-γ-Eudesmol	1620	0.75± 0.20	0.72± 0.21	1.06± 0.01
γ-Eudesmol	1628	0.64± 0.01	-	0.89± 0.01
Cadinol-epi-α	1635	0.32± 0.04	-	0.71± 0.01
α-Cadinol	1648	0.32± 0.05	1.67± 0.05	0.71± 0.01
β-Eudesmol	1653	0.43± 0.02	-	0.89± 0.01
α-Eudesmol	1654	5.14± 0.07	8.37± 0.35	9.74± 0.11

6-epi-Shyobunol	1680	6.75± 0.21	12.32± 0.43	12.39± 0.21
Shyobunol	1689	0.06± 0.01	-	0.53± 0.01
Farnesol, (2Z,6Z)-	1696	0.02± 0	-	0.11± 0.01
Geranyl tiglate	1704	-	-	0.09± 0.02
Farnesol, (2E,6Z)-	1709	-	-	0.05± 0.01
Isoledene	1720	0.86± 0.02	1.08± 0.07	0.53± 0.01
Chamazulene	1727	0.05± 0	-	0.35± 0.02
cis-Z- α -Bisabolene epoxide	1739	0.14± 0.01	-	0.35± 0.01
Benzyl benzoate	1759	0.17± 0.01	-	0.53± 0.01
Tetra-decanoic acid	1769	0.09± 0	-	0.18± 0.02
Ethyl tetra-decanoate	1787	0.18± 0.02	-	0.09± 0.01
Nootkatone	1814	0.04± 0.01	-	0.19± 0.01
Farnesyl acetate	1841	1.50± 0.01	-	1.95± 0.33
Benzyl salicylate	1861	0.03± 0.01	-	0.11± 0.01
1-Hexadecanol	1878	0.05± 0.1	-	0.11± 0.01
2-Heptadecanone	1904	0.04± 0.1	-	0.09± 0.01
Methyl Hexa-decanoate	1931	-	-	0.18± 0.00
Hexadec-9-enoic acid	1956	0.02± 0.01	-	0.04± 0.00
Total concentration%		99.83 ± 0.34	100.00±0.00	99.77± 0.25
Monoterpene hydrocarbons		30.42± 0. 12	2.04± 0.01	9.10± 0.01
Monoterpene alcohols		1.50± 0.01	-	1.41± 0.02
Phenolic monoterpenes		0.88± 0.05	-	0.71± 0.00
Monoterpene ketones		0.07± 0.01	-	-
Monoterpene aldehydes		0.09± 0.01	-	0.11± 0.00
Sesquiterpene hydrocarbons		31.35± 0.09	38.29± 0.02	32.92± 0.01
Sesquiterpene alcohols		31.61± 0.23	58.35± 0.8	49.29± 0.33
Sesquiterpene ketones		0.58± 0.01	1.32± 0.09	1.25± 0.02
Sesquiterpene aldehydes		-	-	-
Others		3.33± 0.01	-	4.74± 0.03
*Note: Compounds were analyzed on HP-5MS column according to C_7-C_{30} n-alkanes standard. -: Not identified				

3.2.5 Significance of major compounds identified in *S. molle* essential oils

Several studies have reported α-phellandrene as one of *S. molle* essential oil's major constituents [2, 8, 9]. α-Phellandrene has been reported to have anti-fungal and antibacterial activities against *Penicillium cyclopean* fungus and *Bacillus* bacteria species [26]. This compound also has antioxidant activities and boost immune system in mice [27]. Furthermore, *S. molle* essential oil in this study is rich in elemol (11.5%, 8.6% and 20.1% in HD, UAHD at 300 W and UAHD at 50 W respectively). Elemol has a green woody sweet odour, and it is known to have significant insecticidal activities [28], such as yellow fever mosquito [29] and ticks repellents [30]. Elemol is well known as a fragrance material in many cosmetic products [31].

Aromadendrene is also one of the major compounds found in the oil (8.5%, 5.6% and 8.2% in HD, UAHD at 300 W and UAHD at 50 W, respectively). Aromadendrene has anti-fungal, antiviral, and antibacterial properties [32, 33]. Another major compound is 6-epi-shyobunol with an abundance of 6.3%, 7.0% and 10.3% in the essential oil extracted by HD, UAHD at 300 Wand UAHD at 50 W respectively. Studies have reported that 6-epi-shyobunol and α-eudesmol (4.8%, 5.5%, 7.0%) have significant antimicrobial properties [34, 35].

Another substantial major compound is limonene in the essential oil extracted by HD (13.92%). This compound has previously been reported as a major compound (16.19% on *S. molle* essential oil [10]. Limonene finds its application as a fragrance and flavouring agent in products such as soap, perfumes, food, and chewing gums [36, 37]. It is also regarded as a safe compound that can relieve heartburn, dissolve cholesterol, and has chemo-preventive effects against various cancers [36, 37].

3.3 Antioxidant activity of *S. molle* essential oil as measured by % of inhibited DPPH free radicals by the essential oil

The IC_{50} for the essential oil extracted by HD is found to be 134.4 µg/ml. In comparison, the IC_{50} for ascorbic acid is found to be 218.3 µg/ml, which implies low antioxidant activity compared to the essential oil. The IC_{50} determined for this essential oil is showing a better antioxidant activity than what is reported in other literature. For example, Abir *et al.* (2016) reported the IC_{50} of 3586.9 mg/L ~ (3586.9 µg/ml) [10], Martins *et al.* (2014) reported an IC_{50} of 800 µg/ml [2]. Although the *S. molle* essential oil in Botswana has better antioxidant activity, Diaz *et al.* (2008) reports a higher antioxidant activity **(**IC_{50} of 0.0363 µg/ml) [11]. The essential oil's antioxidant activity is hinged upon the chemical composition which is highly influenced by various environmental factors.

The IC_{50} for the essential oil extracted by UAHD at 300 W is determined to be 108.8 µg/ml, which is lower than that of Ascorbic acid (218.3 µg/ml), HD (134.4 µg/ml), and UAHD at 50W (226.0 µg/ml). As shown in Table 2, some compounds that are not present in HD and UAHD at 50 W are present in UAHD at 300 W, for example, cis-Z- α-bisabolene epoxide and shyobunol. This may suggest the reason why the essential oil extracted by UAHD at 300 W has better antioxidant activity than that of HD and UAHD at 50 W. Some compounds in this essential oil are also present with higher concentration than those obtained by HD. Compounds in the essential oils work in a synergy to produce significant antioxidant activity properties [38, 39].

The IC_{50} value of the essential oil extracted by UAHD at 50 W is higher (226.0 µg/ml) compared to that of HD and UAHD at 300 W. This signifies that it has low antioxidant activity than ascorbic acid (218.0 µg/ml) and the essential oil extracted by HD and UAHD at 300 W. The essential oil extracted by UAHD at 50 W has few compounds (23 compounds), suggesting why the essential oil has the lowest

antioxidant properties. Furthermore, oxygenated compounds are few in this essential oil compared to the essential oil extracted by HD and UAHD at 300 W. Studies have revealed that oxygenated compounds play a significant role in the antioxidant activity [38, 39].

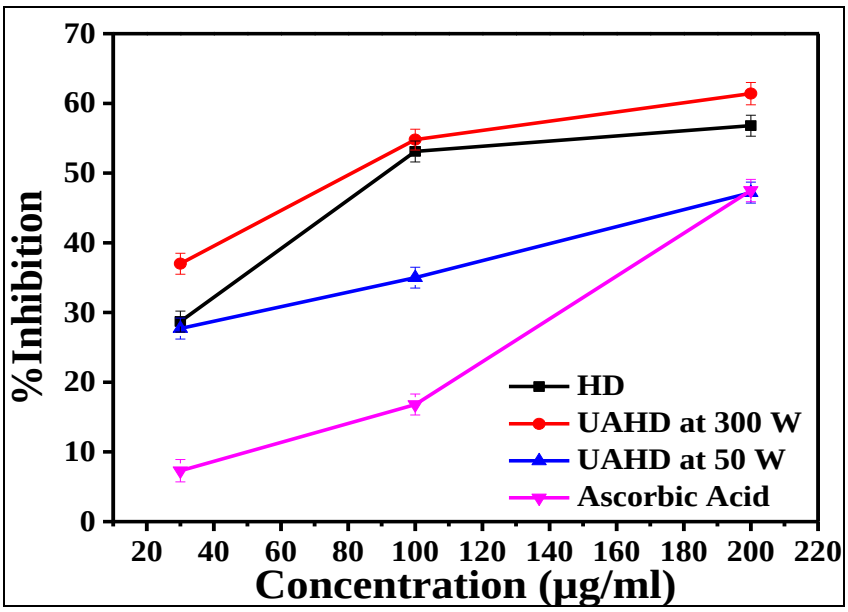

Fig 5: DPPH radical-scavenging activity of the *S. molle* essential oils

4. Conclusion

This study successfully determined the effects of HD and UAHD on the percentage yield, chemical composition, and antioxidant activity of the essential oil from the *S. molle* plants cultivated in Botswana. The essential oils in this study have comparable and, in some cases, better percentage yield than essential oils reported in literature. The chemical compositions and antioxidant activity of the essential oils in this study revealed that the *S. molle* plant has a potential in medicinal chemistry such as anti-inflammation, antibacterial, antifungal, insecticidal, cancer chemo-prevention, heartburn treatment, and dissolving cholesterol.

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6. Declaration of Interest

No declaration of interest.

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