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Chemical composition, antimicrobial and antioxidant activity of *Schinus molle* essential oil from Palapye

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Abstract- Plants have been a source of medical relief for diseases and ailments for millennia, and among this large list of medicinal plants includes the Peruvian pepper tree (Schinus molle). This species produces various compounds with antibacterial properties, such as alkaloids, flavonoids, phenols, and terpenes. This study was aimed at investigating the chemical composition, antioxidant, and antimicrobial activity of S. molle essential oil extracts collected from Palapye on bacterial and yeast colonies as a means to determine the antimicrobial efficacy. Fruit and leaf essential oils of S. molle were extracted using ultrasonic-assisted hydrodistillation. Using gas-chromatography mass spectrometry (GC-MS), 19 compounds were identified from fruit essential oil while only two were identified from the leaf essential oil. Antioxidant activity measured by 2,2-diphenyl-1-picryl-hydrazyl (DPPH), 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and total phenolic content (TPC) showed significantly higher antioxidant activity in the fruit essential oil as compared to the leaf counterpart. Furthermore, antimicrobial activity of the essential oils was determined against bacterial colonies of Staphylococcus aureus and Escherichia coli, and the fungi C. albicans using agar disc diffusion assay. Our results showed one-third efficacy against bacteria as compared to the commercial broad-spectrum antibiotic Gentamicin, while there was no antibiotic activity observed against the fungi.

Keywords*: Antioxidant, antimicrobial, Gas chromatography-mass spectrometry, essential oil, *Schinus molle

I. INTRODUCTION

Antibacterial resistance of pathogenic microorganisms has become a global dilemma, leading to large number of diseases and deaths [1]. Drug resistance patterns observed in various types of bacteria have led to infectious cases that are virtually untreatable by commonly applied antimicrobial methods [2]. This is mainly caused by the excessive, and at times, unnecessary, use of broad-spectrum antibiotics, as the identification and customized treatment plans for specific microbial infections is greatly lacking in most healthcare settings [1]. Bacterial resistance to antibiotics and the development of resistant bacteria have been increasing exponentially, causing many tried and tested treatments to be rendered ineffective against such diseases-causing microorganisms. This has led to the need to develop new, revolutionary treatments [3]. Plants, including the Peruvian pepper tree (*Schinus molle*), produce various compounds with antibacterial properties, such as alkaloids, flavonoids, phenols, and terpenes, known to target the cell membrane of different microorganisms as their bactericidal mechanism of action. It has been recently established that essential oils, when administered at varying concentrations, respond to microbial action in different ways. For example, through blockage or interruption of bacterial communication, known as quorum sensing [4].

A. Distribution, properties, and uses of Schinus molle

The Peruvian or Californian pepper tree (*Schinus molle*) is an evergreen shrub native to Peru and has become widespread in many parts of the world [5]. *S. molle* is a member of the Anacardiaceae family,

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comprising of over 30 species that are native to Central and South America [6, 7]. *S. molle* is commonly used as traditional medicine in South America because of its antifungal, analgesic, antitumoral, antispasmodic, and topical antiseptic properties. It has also been used to treat hypertensive disorders, wounds, asthma, and septic infections [5, 8, 9]. The success of this species' distribution worldwide is credited to its resilience against drought and heat, ability to outcompete native species for nutrients and light, and its quick growth rate and high seed proliferation [10, 11]. The *S. molle* tree has been used as an ornamental plant in Southern African countries including Mozambique, Malawi, South Africa, Botswana, Zambia, and Zimbabwe [12, 13]. The pepper tree, locally known as "Pepere" in Botswana, grows up to between 3-15 m tall, with dark brown, deeply fissured bark. Its leaves are imparipinnate, possessing long, thin leaves, present in clusters of 20-40 leaflets [14]. It produces an edible fruit which, when ripe, is about 5 mm in diameter and often pink-reddish to red in color. Thus far, there are limited to no reports in literature regarding the chemical composition, antioxidant, and antimicrobial activity of *S. molle* from Botswana on bacterial and fungal colonies to combat pathogenic microorganisms. Considering the connection between chemical composition, antioxidant activity, climatic and geographical factors, this study aimed to investigate the chemical composition, antioxidant activity and antimicrobial activity of *S. molle* essential oils (extracted from fruits and leaves) from Palapye on bacterial colonies of *Staphylococcus aureus, Escherichia coli* and the yeast *Candida albicans*. Previous research on *S. molle* has shown that essential oil composition can be greatly affected by soil type, harvest time and the extraction method used [15, 16].

II. METHODOLOGY

A. Sample and extraction of essential oil

Leaves and fruits of female *Schinus molle* were collected in the Palapye area of Botswana. Plant fruits and leaves were later cleaned and dried using liquid nitrogen, and afterwards ground to powder and stored at 4°C until further analysis.

Extraction was done as previously described by Jadhav et al. [17]. About 100 g of powder samples in triplicates was mixed with 300 mL of water and sonicated for 1 hour at 40°C. After an hour, the samples were taken to a simple distillation apparatus set at 100°C and oils were obtained after 6 hours. Essential oils were collected and kept at 4°C until further analysis.

B. Chemical composition, antimicrobial and antioxidant analyses

1. GC-MS analysis of the essential oils

Chemical composition analysis of the essential oils from the fruits and leaves of *S. molle* were performed on gas chromatography-mass spectrometry (GC-MS), with an Agilent GC System 7890B, MSD 5977A, using a 30 m long capillary column (30 m × 0.25 mm × 0.25 µm, calibrated). The carrier gas used was helium at 11.1 psi, at constant pressure and the split ratio was 1:10. The column temperature was programmed from 70°C to 300°C at 5°C/min, with no holding temperature. The fruit essential oil samples were dissolved in n-hexane at a ratio of 1:100 µL; leaf essential oil samples were dissolved at a ratio of 1: 50 µL. Duplicate samples of the essential oils were prepared for GC-MS. Sample volumes of 1 µL of each oil sample were injected manually at a split ratio of 1:10. Chemical components of the essential oils were identified through comparison of their mass spectra and retention indices contained in the NIST MS Search 2.0 (Contributor: NIST Mass Spectrometry Data Centre, 1990).

2. Antioxidant activities: DPPH radical scavenging assay

The free radical scavenging activity of each essential oil was measured using a 2,2-diphenyl-1-picryl-hydrazyl (DPPH) assay as previously reported by Stankovic et al. [18]. Shortly, 0.1 mL of the oil was vortexed vigorously with DPPH solution (60 µM) (in triplicates). The mixture was incubated in the dark for 30 minutes at room temperature and absorbance was measured against blank at 517 nm. The DPPH was presented as mg ferulic acid equivalence/mL of the oil sample (mg FAE/mL of oil sample).

3. Antioxidant activities: ABTS radical scavenging assay

The free radical scavenging activity of each sample was also determined using 2, 2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) solution following a well-established method [19]. Shortly, 0.1 mL of the oil was vortexed vigorously with ABTS solution (in triplicates). The mixture was then incubated in the dark for 30 minutes at room temperature and absorbance was measured against a blank at 735 nm. The ABTS was presented as mg

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ferulic acid equivalence/mL of the oil sample (mg FAE/mL of oil sample).

4. Antioxidant activities: Total phenolic content

Total phenolic content (TPC) was assessed as described previously [20]. In short, 2.5 mL Folin-Ciocalteu reagent (10 folds) and 2.5 mL sodium carbonate (60 g/L) were added to 0.5 mL of each oil extract (in triplicates). The mixtures were incubated in the dark for 15 minutes at 45°C and absorbance was measured against a blank at 765 nm. The TPC was presented as mg ferulic acid equivalence/mL of the oil sample (mg FAE/mL of oil sample).

5. Antimicrobial activity assay: Agar disc diffusion method (Kirby-Bauer Test)

To determine the antimicrobial activity of *S. molle* essential oil extracts, an agar disc diffusion method was conducted. Agar plates containing Mueller-Hinton agar (MHA) for bacterial cultures of *S. aureus* and *E. coli*, and yeast extract peptone dextrose (YPD) for yeast cultures of *C. albicans* were prepared, autoclaved and poured into Petri dishes and stored before use. All microorganisms used were cultured in Mueller-Hinton broth (MHB) at 37°C for 18 hours before use in a VWR 3500 Incubating Orbital Shaker (12620-948, VWR International, Radnor Township, Pennsylvania, USA) at 160 rpm, after which the microbial suspensions were diluted with MHB until a McFarland turbidity standard of 0.5 was achieved. Sterile discs were made from Whatman No. 3 filter paper (6 mm discs) and imbibed with 10 µL of pure essential oils and placed onto the MHA and YPD plates using sterilized forceps. For the bacterial samples, a positive control of Gentamicin antibiotic disc (10 µg/disc), was chosen with 10 µL discs of Mueller Hinton Broth (MHB) used as negative controls. The positive control for yeast was 10 µL discs of cycloheximide 200 µg/mL (prepared from ultra-pure grade crystalline cycloheximide), with the same negative control used as in the antibacterial assays. Commercially sold eucalyptus oil (purchased from a local pharmacy) was used as a secondary positive control as it is a well-established antibacterial [21] and antifungal essential oil [22]. The agar plates were incubated at 37ºC for 24 hours and antimicrobial activity was determined by measurement of the inhibition zones (in millimeters). Measurements for each oil, positive and negative controls were done in triplicates.

C. Statistical analysis

Results were subjected to one-way analysis of variance (ANOVA) by social survey research information (SSRI) statistical software-excel statistics. Values are represented as means ± standard error (SE). Statistical significance of the difference among means was estimated at $P < 0.05$, using Tukey-Kramer's multiple range test. Graphical representation of data was done using OriginPro 8.

III. RESULTS AND DISCUSSION

A. GC-MS analysis: chemical composition of the essential oils

Twenty-one compounds were identified in total, with 19 compounds identified in the fruit essential oils (Table I) and only two identified in the leaf essential oils (Table II). Monoterpenoids (D-limonene, β-pinene, α-phellandrene, β-phellandrene, and ascaridole,) and sesquiterpenoids (caryophyllene, humulene, α-muurolene, tau-cadinol, tau-muurolol, α-cadinol and 6-epi-shyobunol) formed a significant fraction of the *S. molle* fruit essential oil, and the monoterpene β-phellandrene in the leaf oil.

Found in the essential oils of many plants, including culinary staples such as fruits, vegetables, and herbs, monoterpenes are known to be effective in the treatment of several cancers in the early and advanced stages, including mammary, lung and liver cancers, having been used to treat breast and pancreatic carcinomas in mice [23]. Monoterpenes have also been shown to be of great use in the preservation of tropical fruits from fungi [24], and also possess strong antibacterial and antimicrobial activity, with research showing bactericidal and antimicrobial activities of β-pinene, limonene, and α-phellandrene, to name a few [25-28]. Sesquiterpenoids are relevant secondary metabolites found in the natural world, mostly in volatile plant oils, microbes, and some insects [29]. While there has been extensive research in the use of plants in traditional and folk-medicine, very few findings exist with regards to sesquiterpenoids. Among those that do, however, show that sesquiterpenoids are crucial in the treatment of various bacterial and fungal infections [30, 31].

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TABLE I: THE COMPOSITIONS OF THE FRUIT ESSENTIAL OIL EXTRACTS FROM *S. MOLLE* L.

No.	Retention Time (RT)	Name
1	3.11	o-Cymene
2	3.236	D-Limonene
3	3.865	β-Pinene
4	4.123	α-Phellandrene
5	5.559	Octanoic acid, methyl ester
6	8.941	Ascaridole
7	12.528	Cyclohexane, 1-ethenyl-1-methyl-2,4 bis(1-methylethenyl)-
8	12.677	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1α, 2β, 4β)]-
9	12.963	1H-Cycloprop(e)azulene, 1a,2,3,4,4a,5,6,7b-octahydro-1,1,4,7-tetramethyl-, [1aR-(1aα,4α,4aβ,7bα)]-
10	13.209	Caryophyllene
11	14.056	Humulene
12	14.954	1H-Cycloprop(e)azulene, decahydro-1,1,7-trimethyl-4-methylene-
13	15.201	α-Muurolene
14	15.538	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1α, 4aβ, 8aα)-
15	15.859	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-
16	18.525	.tau.-Cadinol
17	18.554	.tau.-Muurolol
18	18.845	α-Cadinol
19	19.675	6-epi-shyobunol

TABLE II: THE COMPOSITIONS OF THE LEAF ESSENTIAL OIL EXTRACTS FROM *S. MOLLE* L.

No.	Retention Time (RT)	Name
1	4.054	β-Phellandrene
2	22.525	Isobutylamine

B. Antioxidant activities: DPPH radical scavenging assay

DPPH antioxidant activities of leaf and fruit essential oils from *S. molle* in Palapye are shown in Figure 1. Free radical scavenging activity of DPPH is based on electron transfer, that produces a violet solution in alcohols such as methanol and ethanol [32]. In the presence of an antioxidant molecule, the free radicals in DPPH experience a reduction of electrons, causing a colourless solution. The stronger the antioxidant, the higher its free radical scavenging activity, which is used to represent the free radical reduction activity of antioxidants [32]. From the standard calibration curve against ferulic acid (linear with $y = 1.1145x + 0.0354$, $R^2 = 0.9295$), the scavenging capacity of the leaf and fruit essential oils of *S. molle* were determined to be 142.8 ± 1.80 mg and 139.4 ± 3.74 mg of ferulic acid equivalent (FAE)/g of sample, respectively (*Fig. 1*). Our data shows significantly high antioxidant activity (value; $P < 0.05$) in leaf essential oil extract when compared to fruit essential oil extract from *S. molle*. While it was expected that the fruit oil extracts should possess higher antioxidant activity, previous research has shown that higher antioxidant activity in leaves may be attributed to the presence of hydroxyls group in oxygenated sesquiterpenes found in the leaf oils [5], which may have not been identified in our GC-MS.

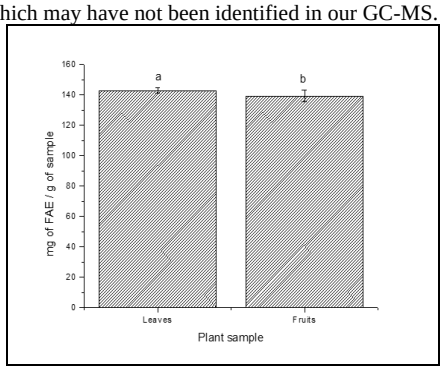

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Fig. 1. DPPH scavenging activity (ferulic acid equivalent) per gram of sample. Values are mean ± standard error (n = 3). Significant differences among the extracts are indicated by different letters (P < 0.05, Tukey-Kramer's range test).

C. *Antioxidant activities: ABTS radical scavenging assay*

The ABTS antioxidant assay measures the scavenging ability of antioxidants in ABTS generated in the aqueous phase. Generation of ABTS occurs by reacting the ABTS salt with potassium persulfate, which is a strong oxidizing agent [33]. The antioxidant activity of *S. molle* using ABTS was determined from the standard calibration curve (linear with y = 0.6733x + 0.159, R^2 = 0.9554) to be 128.1 ± 9.74 and 211.2 ± 33.9 mg of ferulic acid equivalent (FAE)/g of sample for leaf essential oil extracts and fruit essential oil extracts, respectively (*Fig. 2*). Our data shows significantly high antioxidant activity (value; P < 0.05) in leaf essential oil extracts when compared to fruit essential oil extracts from *S. molle*. While it was expected that the fruit oil extracts should possess higher antioxidant activity, previous research has shown that higher antioxidant activity in leaves may be attributed to the presence of hydroxyls group in oxygenated sesquiterpenes found in the leaf oils [5], which may have not been identified in our GC-MS.

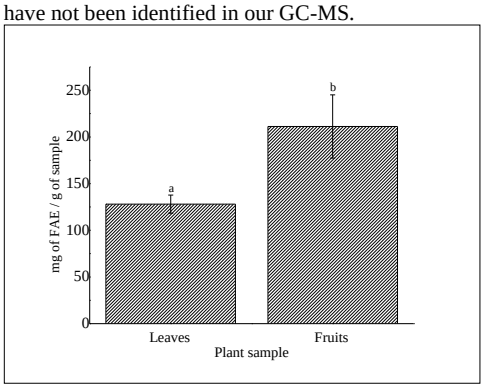

Fig 2. ABTS free-radical scavenging activity (ferulic acid equivalent) per gram of sample. Values are mean ± standard error (n = 3). Significant differences among the extracts are indicated by different letters (P < 0.05, Tukey-Kramer's range test).

D. *Antioxidant activities: Total phenolic content*

The total phenolic content of the essential oils, calculated from the standard calibration curve against ferulic acid (linear with y = 0.9911x + 0.0524, R^2 = 0.9987), were determined to be 250.9 ± 13.5 mg of ferulic acid equivalent (FAE)/g of sample for fruits, while no phenolic content was found in the leaves (*Fig. 3*). Samples were run in triplicates. Phenolic compounds are known to possess reductive-oxidative (redox) properties, granting free radical scavenging capabilities, and permitting them to function as antioxidants [34]. The absence of results in the leaf essential oils may be due to the organic compounds found in the leaves not being phenolic compounds (β-phellandrene and isobutylamine). The results obtained from the fruit essential oils, however, suggest that ultrasonic-assisted hydrodistillation is very effective at extracting phenolic compounds from the fruits of *S. molle*, with a relatively high yield of total phenolic compounds obtained, as displayed by our GC-MS results.

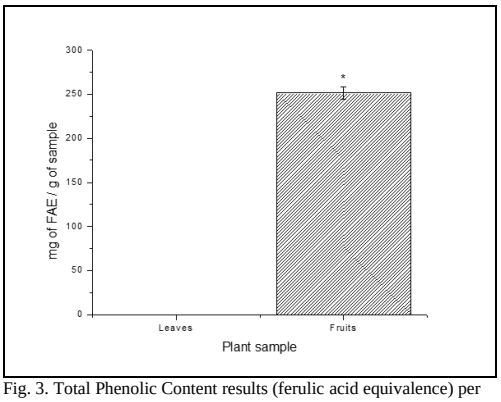

Fig. 3. Total Phenolic Content results (ferulic acid equivalence) per gram of sample. Values are mean ± standard error (n = 3). Significant differences among the extracts are indicated by different letters (P < 0.05, Tukey-Kramer's range test).

E. *Antimicrobial activity assay: Agar disc diffusion method (Kirby-Bauer Test)*

The antimicrobial activity of the fruit essential oil extracts showed promising antibacterial potency against Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria, with no effect observed in yeast (*C. albicans*). The Gentamicin positive control yielded inhibition zones of (on average) 23.3 ± 0.33 mm and 18.7 ± 0.33 mm in *E. coli* and *S. aureus*¸ respectively, with the corresponding inhibition zones of the fruit essential oil extracts being 7.7 ± 0.33 mm and 6.0 mm (*Fig. 4-7*). According to Hosni et al. [35], if the inhibition zone is the size of the disc, i.e., 6 mm, the antimicrobial is considered inactive. These results suggest that pure essential oil extracts from *S. molle* fruits found in Palapye are 33% as effective as the broad-spectrum antibiotic Gentamicin, showing promising potential for their use as antibacterial treatments in medicine and food processing, and plant metabolites have been documented to possess multi-target activity which, unlike commercial antibiotics, does not result in antibiotic resistance [36].

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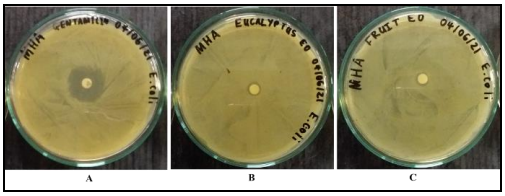

Fig. 4. Agar disc diffusion assays of *E. coli* – Gentamicin 10 µg with an inhibition zone of 23 mm (A); eucalyptus essential oil with an inhibition zone of 8 mm (B); and *S. molle* fruit essential oil with an inhibition zone of 8 mm (C).

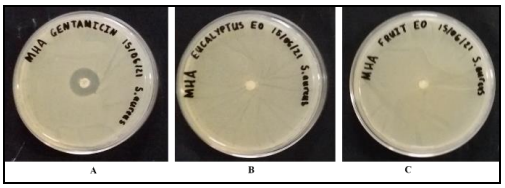

Fig. 5. Agar disc diffusion assays of *S. aureus* – Gentamicin 10 µg with an inhibition zone of 18 mm (A); eucalyptus essential oil with no inhibition zone (B); and *S. molle* fruit essential oil with an inhibition zone of 6 mm (C).

The leaf essential oil extracts did not induce any inhibition, as was also seen by the eucalyptus oil control samples in *S. aureus*¸ although an average inhibition zone of 8 mm was recorded in *E. coli* assays by eucalyptus essential oil. No inhibition of growth was observed in *C. albicans*.

The lack of antimicrobial and antioxidant activity in leaf essential oil extracts could perhaps be attributed to seasonal variations as well as soil composition found in Palapye, as several researchers have already established that *S. molle* leaf essential oils do possess antioxidants and antimicrobial activities of high efficacy [5, 35, 37]. Alternatively, due to the high volatile nature of essential oils, the oils found in the leaves may have volatilized in the 100ºC hydrodistillation setup, leaving us with fewer organic compounds than were initially present.

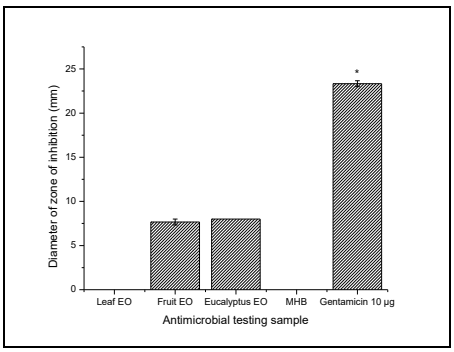

Fig. 6. *E. coli* antimicrobial activity. Values are mean ± standard error (n = 3). Significant differences among the extracts are indicated by an asterisk (P < 0.05, Tukey-Kramer's range test).

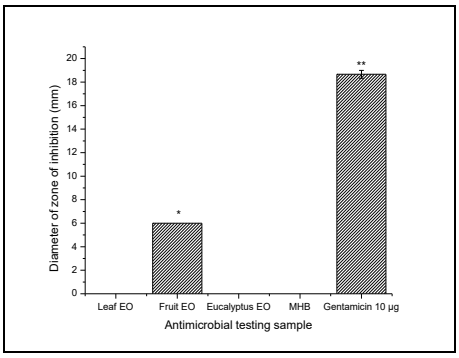

Fig. 7. *S. aureus* antimicrobial activity. Values are mean ± standard error (n = 3). Significant differences among the extracts are indicated by an asterisk (P < 0.05, Tukey-Kramer's range test).

IV. CONCLUSIONS

The essential oil extracts from *S. molle* found in Palapye were successfully extracted and their chemical compounds were identified and analyzed. Qualitative analysis of *S. molle* fruit essential oil extracts have shown promising antimicrobial potential against Gram-positive *S. aureus* and Gram-negative *E. coli*, functioning at approximately one-third the bactericidal capacity of commercial broad-spectrum antibiotics (Gentamicin 10 µg). The antioxidant activities of this plant species found in Palapye have also been established, with results showing a high phenolic content, which are attributed to the distinctive aroma which *S. molle* is well known for, and may be responsible for the antimicrobial activities observed by the Kirby-Bauer tests. The use of medicinal plants to treat a variety of ailments has been a worldwide practice for many generations, and even after the discovery, mass production and distribution of modern-day broad-spectrum antibiotics, still hold a relevant place in the field of medicine and pharmacology. While this research has shone light on the fact that *S. molle* essential oil extracts from trees found in Palapye do possess antimicrobial and antioxidant activities, quantitative work is further required to establish the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of these oils, as well as research on *S. molle* samples obtained from different points of the country and at different seasons to assess efficacy as influenced by environmental and seasonal variations. The use of alternative extraction techniques may also prove to yield much better results, such as hydrodistillation by means of a Clevenger-type apparatus.

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