BIOACTIVE CONSTITUENTS AND POTENTIAL ANTIBACTERIAL ACTIVITY OF LAVENDER TREE (*HETEROPYXIS NATALENSIS* HARV.) LEAVES

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Abstract. *Heteropyxis natalensis* is a South African medicinal tree that has been utilized by Venda and Zulu communities to treat several health ailments. The aim of this study was to elucidate the secondary metabolites in leaf extracts as well as to investigate the chemical profile and antibacterial efficacy of the methanolic leaf extract of *H. natalensis.* Qualitative phytochemical screening confirmed the presence of carbohydrates, alkaloids, phenolics, saponins, sterols, terpenes and fixed fats and oils in leaf extracts. Gas chromatography-mass spectrometry identified 119 bioactive chemical compounds in the methanolic leaf extract of which 14 compounds had a peak area greater than 1%. In addition, methanolic extract was subjected to antibacterial screening using the well diffusion method. *Heteropyxis natalensis* exhibited antibacterial efficacy against 5 strains of pathogenic bacteria*: Escherichia coli, Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus, Pseudomonas aeruginosa* and *Bacillus subtilis.* The results obtained in this study indicate the presence of naturally occurring bioactive compounds in *H. natalensis* leaves.
Keywords: *lavender tree, GC-MS, herbal drug, phytochemical, methanolic extract*

Introduction

The utilization of plant material as a form of medicine is a 60,000-year-old tradition (Qazi and Molvi, 2016) that is still practiced worldwide to treat several health ailments (Meena et al., 2009; Mahomoodally, 2013). Traditional herbal medicine or ethnomedicine forms the backbone of health care in many developing countries plagued by poverty and limitations on modern medical treatment (Koduru et al., 2007; Meena et al., 2009). As a result, ethnomedicine is utilized daily as plant material is inexpensive, moreover, fewer side effects are exhibited (Gunjan et al., 2015; Akinyemi et al., 2018). Herbal medicine is embedded in the socio-cultural lifestyle of third-world African countries (Koduru et al., 2007). Indigenous knowledge on herbal medicine has been formulated through trial and error for hundreds of years and passed on through generations via verbal communication (Gurib-Fakim, 2006; Mahomoodally, 2013).

South Africans rely on herbal medicine as opposed to Western medical treatment since it is affordable, effective and easily accessible (Xego et al., 2016; Akinyemi et al., 2018). Therefore, the South African medicinal plant industry continuously thrives as it is supported by 27 million local consumers (Petersen et al., 2017). However, the indigenous biodiversity of medicinal flora faces several threats such as population

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increase, overharvesting and land degradation (Street and Prinsloo, 2012) resulting in an increased demand for medicinal plant material (Sen and Samanta, 2014; Xego et al., 2016; Akinyemi et al., 2018). Therefore, medicinal plants are phytochemically screened to determine the bioactive chemical compounds responsible for their therapeutic potential (Savithramma et al., 2011) in order to establish preservation management strategies as well as to prevent the overexploitation of important medicinal flora (Sen and Samanta, 2014; Akinyemi et al., 2018). Additionally, screening of medicinal plants is at the forefront of the medical field as the isolated compounds provide a promising future in novel drug development to treat several human diseases such as cancer, diabetes, cardiovascular disease and hepato-renal diseases (Shakya, 2016).

Heteropyxis natalensis (Myrtaceae) is a small, deciduous tree (Adesanwo et al., 2009) inhabiting evergreen forests and bushveld regions ranging from KwaZulu-Natal to Limpopo province (Van Vuuren et al., 2007). It is often called the "Lavender tree" (Adesanwo et al., 2009) as the leaves emit a robust lavender scent (Van Vuuren and Viljoen, 2008). The tree leaves have been utilized by Venda and Zulu communities (Van Vuuren and Viljoen, 2008; Adesanwo et al., 2009) to treat respiratory ailments, nosebleeds, toothaches, bleeding gums and menorrhagia (Van Vuuren and Viljoen, 2008; Henley-Smith et al., 2018). Van Vuuren et al. (2007) reported that the plant is also used as a decongestant and antimicrobial agent. The essential oils of *H. natalensis* have been reported to contain eucalyptol and limonene which are responsible for the species' medicinal characteristics (Gundidza et al., 1993; Van Vuuren et al., 2007). These compounds are used in fragrances, flavors, detergents, cosmeceuticals and pharmaceuticals (Pesonen et al., 2014; Bhowal and Gopal, 2015). The chemical composition and antimicrobial activity of the essential oils of *H. natalensis* have been explored in previous studies (Gundidza et al., 1993; Van Vuuren et al., 2007) but phytochemical and antibacterial investigations of leaf extracts have not been determined. Therefore, this study aimed to elucidate the secondary metabolites in leaf extracts and investigate the chemical profile and antibacterial efficacy of methanolic leaf extract of *H. natalensis*.

Materials and methods

Preparation of leaf extract

Fresh leaves of *H. natalensis* were collected from the University of KwaZulu-Natal, Westville Campus (29°49'01.0"S 30°56'51.2"E; *Fig. 1*) and were air dried at 24°C for 6 weeks. Dried leaves were ground into a fine powder using a domestic spice mill (Kenwood Ltd, United Kingdom) and extracts were prepared by continuous extraction using a soxhlet apparatus. Approximately 10 g of powdered material was added to 100 mL of hexane (organic solvent) in a round bottom flask. The powder and solvent mixture were boiled for three sessions (3 h each) and filtered after each session using Whatman No. 1 filter paper. This process was repeated using chloroform and methanol as solvents. The resulting filtrates were stored in mason jars at room temperature (24°C) and used for phytochemical analyses.

Phytochemical analyses

(a) Detection of carbohydrates

Two drops of Molisch reagent (solution of alcoholic α-naphthol) was added to 2 mL of extract and mixed. Concentrated sulphuric acid (H_2SO_4) (2 mL) was carefully added

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to the solution. The presence of red – violet rings in the middle of the solution indicated the presence of carbohydrates (Vimalkumar et al., 2014).

(b) Detection of alkaloids

Wagner's reagent (1 mL) was added to 2 mL of extract. The formation of a red-brown precipitate indicated the presence of alkaloids (Vimalkumar et al., 2014).

(c) Detection of phenolics

Two drops of 5% ferric chloride was added to 2 mL of extract. Dark green-black precipitate indicated a positive test for phenolic compounds (Mishra et al., 2014).

(d) Detection of saponins

Two mL of extract was diluted with 5 mL of distilled water (dH_2O) and shaken vigorously by hand for 10 min. The persistence of a foam layer for 10 min above the mixture indicated the presence of saponins (Dhanesekaran et al., 2014).

(e) Detection of sterols

Chloroform (2 mL) and 2 mL of concentrated H_2SO_4 was added to 2 mL of extract and shaken carefully for 2 min. Sterols were present if the chloroform layer was red and the acid layer was fluorescent green (Sheel et al., 2014).

(f) Detection of terpenes

Chloroform (2 mL) was added to 5 mL of extract. Concentrated H_2SO_4 (3 mL) was carefully added on the inner side of the test tube. Red – brown rings indicated a positive test for terpenes (Ismail et al., 2016).

(g) Detection of fixed fats and oils

A drop of extract was placed on Whatman No. 1 filter paper and the appearance of an oily residue tested positive for fixed fats and oils in the extract (Mishra et al., 2014).

Figure 1. *Heteropyxis natalensis growing at the University of KwaZulu-Natal (Westville campus)*

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Gas chromatography–mass spectrometry (GC-MS)

Powdered leaf material (±2.5 g) was added to 50 mL of methanol in a round bottom flask. The powder and solvent mixture were boiled for 2 h followed by a 1 h session. Thereafter, the filtrates were filtered using Whatman No. 1 filter paper and stored in a mason jar at 24°C. The filtrate was analyzed using GC-MS (QP-2010 Ultra Shimadzu, Japan) with an Rx-5SilMS fused silica capillary column of 30 m length (0.25 µm internal diameter and 0.1 µm film thickness). Helium was used as the carrier gas with a flow rate of 0.96 $mL.min^{-1}$ and a total flow of 4.9 $mL.min^{-1}$, along with a linear velocity of 36.7 $cm.s^{-1}$ at a purge flow of 3.0 $mL.min^{-1}$. The injection port temperature was set at 250°C. The oven was set to an initial temperature of 50°C and was maintained at this temperature for 1 min. Then, the temperature was increased to 310°C at a rate of $5°C.min^{-1}$ and was maintained at that temperature for a further 10 min. The MS was taken at 70 eV with a mass range of 50 to 800 $m.z^{-1}$. Chemical compounds present in the methanolic extract of *H. natalensis* were identified using NIST Chemistry WebBook (2018) and expressed as percentages based on peak area.

Antibacterial assay

Additional methanolic extract was dried and preserved at 4°C for the antibacterial assay. Screening of antibacterial activity of methanolic extract was determined using the agar well diffusion method described by Saif et al. (2017) against two strains of gram-negative bacteria: *Escherichia coli* (ATCC 25218) and *Pseudomonas aeruginosa* (ATCC 27853) and three gram-positive bacterial strains: *Staphylococcus aureus* (ATCC 25923), methicillin-resistant *Staphylococcus aureus* (MRSA) (Clinical type) and *Bacillus subtilis*. Bacterial strains were grown for 18 h in Nutrient Broth (Biolab, South Africa) in a 37°C incubator on a shaker and were then standardized by adjusting the turbidity of the samples according to a 0.5 McFarland standard by suspending the bacteria in sterile dH2O. The medium was prepared by mixing 38 g Mueller Hinton agar (MHA) in 1 L of dH2O. The medium was then boiled for 1 min and autoclaved at 121°C for 20 min. After reaching room temperature (24°C), the medium was carefully poured into sterile petri dishes and left to solidify under a sterile environment. Each bacterial strain was evenly swabbed onto MHA plates and left to dry. Thereafter, wells were aseptically punched using a sterile cork borer (5 mm in diameter). One gram of dried methanolic extract was resuspended in 1 mL of methanol and 90 µl of the stock solution (1 $mg.mL^{-1}$) was pipetted into each well. Petri dishes were then transferred to incubators of various temperatures to allow the extract to diffuse through the MHA and inhibition zones were observed after 18 h.

Results and discussion

Qualitative phytochemical screening of *H. natalensis* leaves revealed the presence of several classes of secondary metabolites in hexane, chloroform and methanol extracts (*Table 1*). These compounds provide protection against plant damage and disease, deter herbivorous insects as well as withstand harsh environmental conditions (Saxena et al., 2013; Ahmed et al., 2017). In addition to plant defense, the phytochemical constituents presented in *Table 1* play an important role in combatting many human ailments (Wink, 2015; Pakkirisamy et al., 2017). It has been reported that the medicinal potential of a plant is directly proportional to the occurrence of biologically active chemical

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compounds within the species (Saxena e t al., 2013; Pakkirisamy et al., 2017). Alkaloids, terpenes and phenolics exhibit several pharmacological activities such as anticancer, anti-inflammatory, antiulcer and antibacterial activities (Saxena et al., 2013; Shakya, 2016; Aziz and Banerjee, 2018). Medicinal plants containing saponins and sterols display anticancer and antimicrobial properties (Shakya, 2016; Kumar et al., 2018). These compounds were also present in the leaf extracts of other myrtaceous species such as *Syzygium cumini* and *Eugenia uniflora* (Fathima and Pandian, 2015; Aziz and Banerjee, 2018; Kumar et al., 2018). In addition to the medicinal properties, these compounds are also responsible for flavor, color and aroma in food, perfumes and cosmetics (Saxena et al., 2013).

Table 1. *Qualitative phytochemical screening of leaf extracts of H. natalensis*

Phytochemical compounds	Hexane	Chloroform	Methanol
Carbohydrates	+	+	+
Alkaloids	+	++	+
Phenolics	-	-	++
Saponins	+	+	-
Sterols	+	+	+
Terpenes	+	+	+
Fixed fats and oils	+	+	+

- = Absent, + = Present, + + = Intense reaction

The chemical compounds in the methanolic extract of *H. natalensis* were identified by GC-MS analysis. The extract revealed the presence of 119 bioactive compounds. However, among the 119 compounds, 14 of them are reported in *Table 2* as their peak area was greater than 1%. Compounds with a peak area less than 1% were considered as low-level compounds (Pakkirisamy et al., 2017). The main chemical compounds present in *H. natalensis* were squalene (13.57%) and 1,2,3-benzenetriol (22.53%) which were also identified in the following myrtaceous species: *Syzygium jambos* (Sharma et al., 2013) and *Eugenia flocossa* (Kala et al., 2012), respectively. These naturally occurring bioactive compounds in these plants are primarily responsible for their medicinal properties.

The synthesis of squalene occurs in plants and animals and is also a precursor for sterol synthesis (Lozano-Grande et al., 2018). Squalene is an independent oxygen carrier that carries oxygen to various cell membranes in the body experiencing limited levels of oxygen (Gűneş, 2013). The pharmacological properties of squalene include antitumor and antioxidant activity (Gűneş, 2013; Lozano-Grande et al., 2018) which was highlighted in *Table 3*. The leaf paste of *E. flocossa* is used to treat rheumatic pain (Kala et al., 2012). The anti-inflammatory properties of *E. floccosa* leaf extract could be attributed to 1,2,3-benzenetriol (26.72%), the main constituent compound. However, according to *Table 3* and Kala et al. (2012), 1,2,3-benzenetriol does not exhibit the aforementioned pharmacological property. This indicates that a variety of chemical compounds within the plant may work additively or synergistically to bring about a greater effect than an individual compound which may catalyze the healing process (Mahomoodally, 2013). Therefore, the compounds present in *H. natalensis* are working as a cohesive system to treat ailments such as respiratory disorders, menorrhagia and gingivitis (Van Vuuren and Viljoen, 2008; Henley-Smith et al., 2018).

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Table 2. *Phytochemical compounds with a peak area % > 1 in the methanolic extract of H. natalensis detected by GC-MS*

No.	Compound	CAS	RT (min)	Area %	Mol formula	Mol weight
6	Eucalyptol	470-82-6	6.686	5.41	$C_{10}H_{18}O$	154
24	Hydroquinone	123-31-9	10.311	1.17	$C_6H_6O_2$	110
32	1,2,3-Benzenetriol	87-66-1	11.701	22.53	$C_6H_6O_3$	126
38	2-Isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,7-octahydronaphthalene	0-00-0	13.338	1.06	$C_{15}H_{24}$	204
45	1α,2β,3α,5β -Cyclohexanetetrol	53585-08-3	14.887	3.92	$C_6H_{12}O_4$	148
62	9-Octadecen-1-ol, (Z)-	143-28-2	19.292	1.93	$C_{18}H_{36}O$	268
84	(S)-5,7-dihydroxy-6,8-dimethyl flavanone	56297-79-1	23.764	7.81	$C_{17}H_{16}O_4$	284
85	2,2,4-Trimethyl-6-(1-oxo-3-phenylprop-2-enyl)-cyclohexane-1,3,5-trione	0-00-0	23.978	1.25	$C_{18}H_{18}O_4$	298
86	5,7-Dimethoxyflavanone	1036-72-2	24.161	3.82	$C_{17}H_{16}O_4$	284
89	Benzaldehyde, (diphenylmethylene) hydrazine	13118-38-2	24.634	2.21	$C_{20}H_{16}N_2$	284
93	Squalene	111-02-4	25.400	13.57	$C_{30}H_{50}$	410
106	Vitamin E	59-02-9	27.582	3.67	$C_{29}H_{50}O_2$	430
110	β-Sitosterol	83-46-5	29.258	2.17	$C_{29}H_{50}O$	414
117	α-Amyrin	638-95-9	30.265	2.21	$C_{30}H_{50}O$	426

Table 3. *Pharmacological properties associated with the chemical compounds with a peak area % > 1 in the methanolic extract of H. natalensis*

No.	Compound	Properties
6	Eucalyptol	Anti-inflammatory, antioxidant, antiseptic, antimicrobial (Juergens, 2014)
24	Hydroquinone	Anti-oxidant, neuroprotective, immunomodulatory, anti-inflammatory (Byeon et al., 2018)
32	1,2,3-Benzenetriol	Antioxidant, antiseptic, antibacterial, antidermatitic (Kala et al., 2012)
38	2-Isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,7-octahydronaphthalene	Antifungal (Wang et al., 2011)
45	1α,2β,3α,5β-Cyclohexanetetrol	Antioxidant, antimicrobial, anti-inflammatory (Ravikumar et al., 2012)
62	9-Octadecen-1-ol, (Z)-	No activity reported (Karthikeyan et al., 2016)
84	(S)-5,7-dihydroxy-6,8-dimethyl flavanone	Antimalarial (Bero et al., 2009)
85	2,2,4-Trimethyl-6-(1-oxo-3-phenylprop-2-enyl)-cyclohexane-1,3,5-trione	Antimicrobial (Bonilla et al., 2015)
86	5,7-Dimethoxyflavanone	Anti-inflammatory (Panthong et al., 1989)
89	Benzaldehyde, (diphenylmethylene) hydrazine	Anti-bacterial, antifungal, antitumor (Arulkumaran et al., 2017)
93	Squalene	Antibacterial, antioxidant, immunostimulant, antitumor (Karthikeyan et al., 2016)
106	Vitamin E	Antioxidant, anticancer, anti-inflammatory (Rizvi et al., 2014)
110	β-Sitosterol	Antimicrobial, antidiabetic, anti-inflammatory, anticancer, Bin Sayeed et al., 2016)
117	α-Amyrin	Anti-inflammatory (Okoye et al., 2014)

The antibacterial efficacy of *H. natalensis* against 5 bacterial strains is summarized in *Table 4*. The methanolic extract exhibited varying degrees of inhibition against *E. coli*, *P. aeruginosa*, *S. aureus*, MRSA and *B. subtilis*. The highest degree of inhibition was exhibited against MRSA. This strain is known to cause pneumonia, meningitis, urinary tract infections and nosocomial contamination (Heyman et al., 2009). The smallest zone of inhibition was exhibited against *B. subtilis*. This suggests that the strain was less sensitive compared to the other microorganisms used in this study. Liliwirianis et al. (2011) highlighted that a small inhibition zone is attributed to their ability to form

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endospores (resting stage), thus allowing them to remain viable and more resistant over time. According to the results presented in *Table 4*, the leaves of *H. natalensis* can be used as effective antibacterial agents in the treatment of gastrointestinal, skin, respiratory and nosocomial ailments as the phytochemicals present in the leaves (*Tables 1* and 2) exhibit various pharmacological properties (*Table 3*). These results confirm previous findings by Gowri and Vasantha (2010) wherein the methanolic extract of the leaves of *S. cumini* were investigated.

Table 4. *Screening of antibacterial activity of methanolic extract of H. natalensis*

Bacterial strain	Zone of inhibition (mm)[a]
E. coli	9 ± 0.21
S. aureus	8 ± 0.27
P. aeruginosa	8 ± 0.27
MRSA	10 ± 0.32
B. subtilus	7 ± 0.12

[a]Inhibition zone including the cork borer (5 mm) diameter. Data presented are means ± standard error

Conclusions

The results obtained in this study provides substantial information for the utilization of the leaves of *H. natalensis* as traditional medicine. Several phytochemical classes and compounds were elucidated along with their pharmacological activity. In addition, antibacterial screening of the methanolic extract against various strains of bacteria suggests the leaves of *H. natalensis* possess bioactive compounds that could be potentially utilized as antibacterial agents. However, further researches on the antibacterial activity, dose concentration and cytotoxicity are required. The results presented in this study could potentially benefit future research regarding the development of herbal drugs.

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https://researchspace.ukzn.ac.za/server/api/core/bitstreams/1762e441-9aa1-48c6-aab4-e06f8cb9bb7c/content.

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