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ORIGINAL ARTICLE

Gas Chromatography–Mass Spectrometry Analysis of the Volatile Compounds from the Ethanol Extracts of *Bulbine asphodeloides* and *Helichrysum petiolare*

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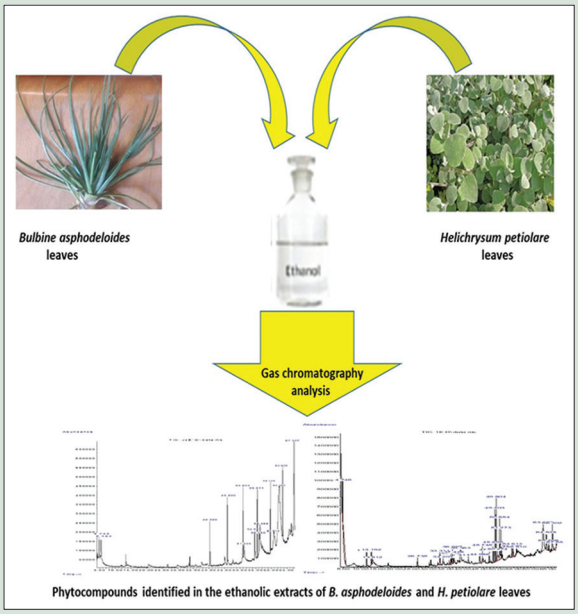
ABSTRACT

Background: *Bulbine asphodeloides* and *Helichrysum petiolare* are important medicinal plants that commonly used in folklore medicine in South Africa for the management of skin ailments such as acne, burns, wounds, eczema, shingles, hives, psoriasis, sores, rosacea, and rashes. Despite their extensive use in traditional medicine, the chemical profiles of these medicinal plants have not been elucidated. **Objective:** The present study was carried out to identify and compare the gas chromatography–mass spectrometry (GC–MS) profiles of the volatile components from the ethanol extracts of both plants. **Materials and Methods:** The fresh leaves of both plants were collected from Alice, Eastern Cape, South Africa, and later oven dried and subjected for extraction and these extracts were further subjected to GC–MS analysis using standard procedures. **Results:** The GC–MS analysis revealed the presence of bioactive terpenes in the volatile components of the ethanol extracts of *B. asphodeloides* and *H. petiolare*. **Conclusion:** The findings reveal the presence of various bioactive compounds which therefore validates the therapeutic importance of these plants in the treatment of skin-related diseases.

Key words: *Bulbine asphodeloides,* gas chromatography–mass spectrometry, *Helichrysum petiolare*, phytochemicals, skin ailments

SUMMARY

- *Bulbine asphodeloides* and *Helichrysum petiolare* are important medicinal plants that commonly used in folklore medicine in South Africa for the management of skin ailments. Our results revealed the presence of bioactive terpenes in the volatile components of both plant which therefore validate their therapeutic importance in the treatment of skin-related diseases. However, we recommend further studies in the field of natural products to isolate, purify, and characterize these bioactive molecules to develop novel drugs in the treatment of skin diseases.



Abbreviations Used: GC-MS: Gas chromatography–mass spectrometry; MSD: Mass selective detector.

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INTRODUCTION

Plant secondary metabolites include a broad variety of phytochemicals (e.g., alkaloids, phenolics, terpenoids, and their derivatives) present in the extracts of different plant parts. Secondary metabolites are believed to provide an evolutionary advantage for the plant species, thereby triggered in response to predators, diseases, and stress. This evolution of defense compounds in plants has given rise to bioactive compounds that may have valuable effects in medicine, pharmacy, and biotechnology. Increasing numbers of novel compounds from several plant species are being identified and investigated for their potential pharmacological use.[1]

Bulbine asphodeloides (L.) Spreng is a small star-shaped flower on a tall square-shaped stem that belongs to the family Xanthorrhoeaceae (formerly *Asphodelaceae*). It is locally called Snake Flower (English); Balsam kopieva (Afrikaans); itswelemyoka (Xhosa); ibhucu (Zulu); and pekane (Sesotho).[2] The plant is widely distributed within South Africa most, especially in the Eastern Cape, Western Cape, Northern Cape, Gauteng as well as Mozambique, Swaziland, and Lesotho in semi-karoid and grassland areas. Conventionally, the leaf

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gel of *B. asphodeloides* is applied into wounds, itches, burn, sunburns, rough skin, and insect bites.[3] In addition, the crushed leaves of the plant are used as a dressing for burns, whereas the leaf juice is applied to the cracked lips.[4,5] Furthermore, the fresh or dried roots of the plant are also taken orally in the form of infusion to suppress diarrhea and vomiting.[6] The Xhosas are known to infuse the root of the plant in milk for the treatment of bilharzia.[7] Despite the traditional usage of this plant, no published *in vitro* or *in vivo* studies are available in the literature.

On the other hand, *Helichrysum petiolare* Hilliard and B.L. Burtt (*Asteraceae* family) are a vigorous shrub which grows up to 6 cm in height. *H. petiolare* is a perennial herb that consists of roundish leaves which are covered with silver-gray hairs.[8] It is commonly known as bedding helichrysum (English); kooigoed (Afrikaans.); imphepho (Xhosa). The plant is distributed in South Africa's provinces of Eastern Cape, Western Cape, and Kwazulu-Natal; in the drier inland parts, on sheltered slopes, and along forest margins. The decoction of the leaves is used conventionally to improve skin texture and beauty,[3] and the leaf juice is widely used on wounds to prevent infections.[9] In addition, the infusion of the leaves is used to treat asthma and chest problems.[3] Pharmacological studies of acetone and methanol extracts of *H. petiolare* have shown that the plant has antimicrobial, antioxidant activities, and anti-inflammatory activities.[10] The chloroform and methanol extract of *H. petiolare* have also been reportedly toxic to Graham cells (transformed human kidney epithelial cells).[11] Some of the phytochemicals compounds earlier reported are phloroglucinol, pyrenes, caryophyllene oxide, spathulenol, and flavonoids.[10] However, there is a dearth of in-depth studies on the detailed chemical composition of the two plants. Hence, the present study was undertaken to identify and compare the gas chromatography–mass spectrometry (GC–MS) profiles of the volatile components from the ethanol extracts of *B. asphodeloides* and *H. petiolare* with the objective of validating their ethnobotanical usage in the treatment of skin diseases.

In this study, *B. asphodeloides* and *H. petiolare* were selected based on two criteria. first, these plants have ethnopharmacological data indicating their traditional usage for the treatment of skin diseases in South Africa[12] and despite their extensive traditional usage, detailed chemical composition, and bioactivity of these plants are yet to be investigated. The second criteria were based on the availability of the plant materials. Based on these criteria, *B. asphodeloides* and *H. petiolare* were then selected for this study.

MATERIALS AND METHODS

Plant collection, identification, and extraction

The leaves of *B. asphodeloides* and *H. petiolare* were collected in August 2018 from Alice (Eastern Cape province of South Africa). The plants were botanically identified and authenticated at the Giffen Herbarium of the University of Fort Hare, where voucher specimens BUR-2024 for *B. asphodeloides* and HEL-1340 for *H. petiolare* were deposited. The leaves of *B. asphodeloides* and *H. petiolare* were detached from the rest of the plant, washed with clean tap water to remove debris and were then oven dried to a constant weight at 30°C for 24 h and further pulverized to a homogeneous powder using an electric blender (Commercial Blender type GB27, Hamilton Beach Brands, Inc. China). Approximately 60 g of the powdered plants were extracted separately in 1000 ml of ethanol (Merck) (99.99%) on an orbital shaker (Labcon laboratory service [Pty], South Africa) for 24 h. The extracts were thereafter filtered using a Buchner funnel and Whatman No. 1 filter paper, and the filtrate was concentrated to dryness using a rotary evaporator (Heidolph Laborata 4000, Heidolph instruments, GmbH and Co, Germany). Thereafter, the extracts were reconstituted to their respective solvents to give the required concentrations used in this study. The yield of the extracts was calculated using the formula:

Percentage (%) Yield = (A/B) × 100

Where A = weight of extract.

B = weight of plant material.

Gas chromatography–mass spectrometry analysis

The GC–MS analysis of the ethanol extracts was carried out using the method described by Ezhilan and Neelamegam.[13] An Agilent 7890 GC system equipped with an Agilent 5977A Mass Selective Detector system (Chemetrix (Pty) Ltd.; Agilent Technologies, DE (Germany) with an Elite-5MS (5% diphenyl or 95% dimethyl polysiloxane) fused a capillary column (30 m × 0.320 mm × 0.250 µm film thickness). GC-grade helium (99.999% purity) was used as a carrier gas at a flow rate of 1 ml/min, and splitless 1 µL injections were used. Thereafter, a needle with the samples was inserted directly into the inlet of the gas chromatograph. Injector temperature, ion source temperature, and pressure were maintained at 250°C, 200°C, and 48.745 kpa, respectively. While the oven temperature started from 110°C (held for 1 min), with an increase of 10°C/min to 200°C, then 5°C/min to 280°C, ending with a 9 min isothermal at 280°C. Mass spectra were acquired at 70 eV, and fragments ranged from 45 to 450 Da.

Identification of components

Identification of the components of the extracts was achieved by comparing their retention times and mass spectra data with those of certified in the National Institute of Standard and Technology 11 library source. The chemical classes of the compounds identified in this study were confirmed using the UCSan Diego Metabolomics Workbench database.

RESULTS AND DISCUSSION

The yields of the ethanolic leaf extracts were 10.8% and 9.9% for *B. asphodeloides* and *H. petiolare,* respectively. The results of the GC/MS analyses of the extracts displayed in Figures 1 and 2, whereas Tables 1 and 2 show the variation in the chemical and relative content of both plants species, respectively. The reported biological activities of some identified compounds are presented in Table 3.

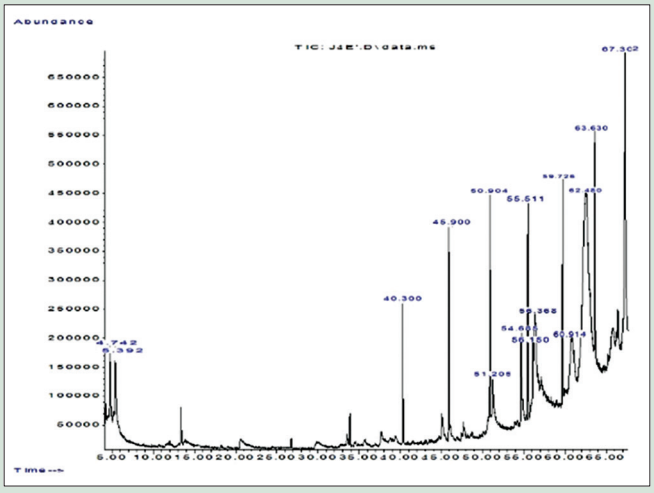

Figure 1: Gas chromatography–mass spectrometry chromatogram of ethanol extract of *Bulbine asphodeloides* leaves

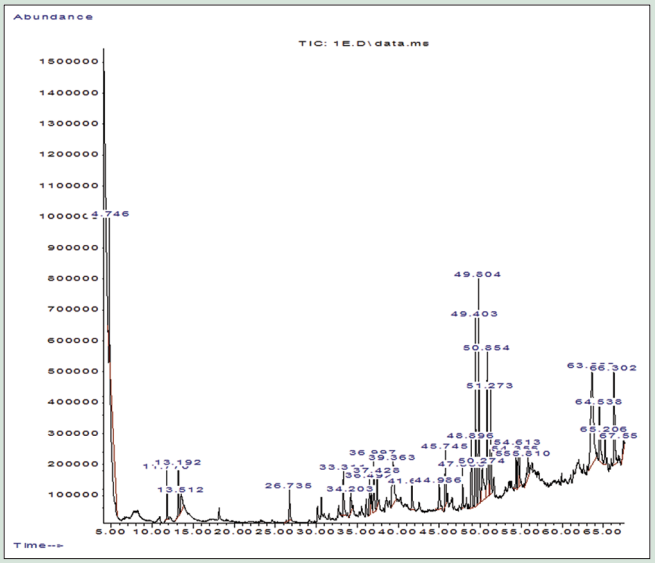

Figure 2: Gas chromatography–mass spectrometry chromatogram of ethanol extract of *Helichrysum petiolare* leaves

A total of 10 compounds were identified in the *B. asphodeloides* extract, whereas 23 compounds were identified in the *H. petiolare* extract.

The GC–MS analysis results revealed the presence of higher percentage of sesquiterpenes (33. 37%) followed by fatty acid esters (12.62%) and diterpenes (8.03%) in the extract of *H. petiolare* than those in *B. asphodeloides* extract. However, monoterpenes, oxides, phenols, and sesquiterpenes class of compounds were not found in the extract of *B. asphodeloides* but detected at a level lower than 2% in the *H. petiolare* extract. The alcohol (39.38%) and alkanes (15.76%) constituents were relatively higher in the extract of *B. asphodeloides* compared to that of *H. petiolare* extract (3.59% and 9.95%, respectively). Whereas, ketone was only detected in a lower percentage in the *B. asphodeloides* extract [Table 1]. However, the literature study shows that there are no available reports with regard to the comparative analysis of chemical profiles of both plants. This is the first study to compare the chemical profiles of both plants using GC–MS.

Among the identified compounds, the cryptomeridiol which is an alcoholic compound has been indicated to exhibit melanogenesis activity, whereas hexadecanoic acid, ethyl ester, has also been reported to function as hair- and skin-conditioning agent.[14] 2,4-Di-tert-butylphenol identified in the extract of *H. petiolare* was reported to demonstrate fungicidal activity against *Aspergillus niger*, *Fusarium oxysporum*,

Table 1: Phytocompounds identified in the ethanolic extract of *Bulbine asphodeloides* leaves by gas chromatography-mass spectrometry

Compound name	Chemical formular	Rt (min)	Area (%)
Hexadecamethylcyclooctasiloxane	$C_{16}H_{48}O_8Si_8$	40.3	6.85
1,1,1,5,7,7,7-Heptamethyl-3,3-bis (trimethylsiloxy) tetrasiloxane	$C_{13}H_{40}$	45.9	3.52
Octadecamethylcyclononasiloxane	$C_{18}H_{54}O_9Si_9$	50.903	5.39
Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	51.205	2.06
Phytol	$C_{20}H_{40}O$	54.685	3.43
Linoelaidic acid	$C_{18}H_{30}O_2$	56.151	2.53
9,12,15-Octadecatrienoic acid, ethyl ester, (Z, Z, Z)-	$C_{20}H_{34}O_2$	56.368	5.86
3a, 9-Dimethyldodecahydrocyclohepta[d] inden-3-one	$C_{16}H_{26}$	60.914	6.3
17-(1,5-Dimethylhexyl)-10,13-dimethyl-4-vinylhexadecahydrocyclopenta[a] phenanthren-3-ol	$C_{29}H_{50}O$	62.48	39.38
N-Benzyl-N-ethyl-p-isopropylbenzamide	$C_{19}H_{23}$	63.63	5.6

Compounds are listed in order of Rt. Rt: Retention time

Table 2: Phytocompounds identified in the ethanolic extract of *Helichrysum petiolare* leaves by gas chromatography-mass spectrometry

Compound name	Chemical formular	Rt (min)	Area (%)
Eucalyptol	$C_{10}H_{18}O$	13.192	2.05
1,5,5-Trimethyl-6-methylene-cyclohexene	$C_{10}H_{16}$	26.735	1.39
(1S,2E,6E,10R)-3,7,11,11-Tetramethylbicyclo[8.1.0]undeca-2,6-diene	$C_{15}H_{24}$	33.311	2.58
2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	34.203	1.04
(-)-Spathulenol	$C_{15}H_{24}O$	36.492	1.89
Ledol	$C_{15}H_{26}O$	36.997	4.9
δ-Selinene	$C_{15}H_{24}$	39.363	5.64
Isoaromadendrene epoxide	$C_{15}H_{24}O$	41.658	1.71
Cryptomeridiol	$C_{15}H_{28}O_2$	44.986	2.06
α-Farnesene	$C_{15}H_{24}$	45.745	2.3
1H-3a, 7-Methanoazulene, octahydro-1,4,9,9-tetramethyl-	$C_{15}H_{26}$	47.835	1.56
Cyclohexanecarboxylic acid, 4-(chloromethyl)-1-methyl-, methyl ester	$C_{10}H_{17}$	49.403	8.12
p-Camphorene	$C_{20}H_{32}$	50.274	4.6
5,5-Dimethyl-1-vinylbicyclo[2.1.1]hexane	$C_{10}H_{16}$	50.854	8.07
Indazol-4-one, 3,6,6-trimethyl-1-phthalazin-1-yl-1,5,6,7-tetrahydro-	$C_{18}H_{18}O_4$	51.273	5.07
Caparratriene	$C_{15}H_{26}$	54.355	1.65
Phytol	$C_{20}H_{40}O$	54.613	2.31
Alloaromadendrene	$C_{15}H_{24}$	63.626	17.33
1-Heptatriacotanol	$C_{37}H_{76}O$	54.807	1.53
Oxonin, 4,5,6,7-tetrahydro-, (Z, Z)	$C_8H_{12}O$	55.81	2.07
2-(N-Phenyl-aminosulfanyl)-benzoic acid methyl ester	NA	64.538	4.5
11-Methyltricosane	$C_{24}H_{50}$	65.206	1.88
Phenol, 2-ethoxy-5-(1,2-dihydroxyethyl)-	NA	66.302	7.77
Hexadeca-2,6,10,14-tetraen-1-ol, 3,7,11,16-tetramethyl-	$C_{20}H_{34}O$	67.555	0.84

Compounds are listed in order of Rt. N/A: Not available in database; Rt: Retention time

Table 3: Reported biological activities of some identified compounds in the extracts of *Bulbine asphodeloides* and *Helichrysum petiolare* leaves

Name of compound	Reported biological activity	References
Eucalyptol	Anti-inflammatory, antioxidant, and antibacterial	[14-16]
2,4-Di-tert-butylphenol	Antifugal and antioxidant	[17]
(-)-Spathulenol	Anti-inflammatory, antioxidant and cell proliferation, immunomodulatory effects, and mosquito-repellent activity	[14,18]
Ledol	Antifugal	[14]
Cryptomeridiol	Antiplasmodial and melanogenesis activity	[14]
p-Camphorene	Anticancer, perfume, antitumor, and antixoidant	[14]
Hexadecanoic acid, ethyl ester	Hair- and skin-conditioning agent and anti-uric acid	[14]
Phytol	Cytotoxic, antinociceptive, antioxidant, antimicrobial, anti-inflammatory, anticancer, and diuretic	[14,18]
Alloaromadendrene	Antifugal	[19]

and *Penicillium chrysogenum* by the disc diffusion assay.[15] Alloaromadendrane identified in the extract of *H. petiolare* has also been reported to be very effective inhibitor of the growth of *Cladosporium herbarium,* a species implicated in the development of skin-related problem.[16] Terpenes are the well-known class of compound for a wide range of biological activities and therapeutic applications against cancer, skin, and inflammatory diseases. In this study, spathulenol (1.04%) and eucalyptol (2.05%) were identified as monoterpene and sesquiterpenes in high amount in the extract of *H. petolare,* whereas phytol, a diterpene detected in the extract of both plants, was reported to play an important role in the dispensation of glucose, thereby helping to restore the metabolic activities of patients suffering from Type 2 diabetes.[17] It has also been reported to show *in vitro* cytotoxic, antinociceptive, antioxidant, antimicrobial, anti-inflammatory, anticancer, and diuretic activities.[17] Spathulenol, one of the major monoterpenes identified in the extract of *H. petiolare,* has been found to exhibit inflammatory and antimicrobial activities.[17] It has also been reported to inhibit the growth of *Staphylococcus aureus* and *Proteus mirabili* which are the major bacteria implicated in skin diseases.[16] Eucalyptol is known to possess anti-inflammatory, antioxidant, and antibacterial activities.[14] This compound also reported to show activity against *S. aureus*.[18] Pattnaik *et al*., in 1997,[19] also reported strong antibacterial activity of eucalyptol against 16 bacterial and seven fungal species. It can be observed from this study that the high percentage of terpenes (43.79%) found in the extract of *H. patiolare* leaves is in accordance with those reported from previous studies, in which, terpenes were the major constituents.[20,21] Therefore, it can be suggested that the lower percentage of terpenes in the ethanol extract of *B. asphodeloides* could impact negatively on their biological activities compared to that of *H. patiolare* extract.

This study, therefore, confirm that ethanol extracts of *B. asphodeloides* and *H. patiolare* leaves are composed of bioactive components which are known to exhibit medicinal values coupled with physiological activities. Therefore, isolation of individual phytochemical components may proceed to find a novel drug or a leading compound.

CONCLUSION

The present study is the first report of the chemical profiling by GC/MS of ethanol extracts of *B. asphodeloides* and *H. patiolare* leaves and it should be noted that GC/MS detects only volatile compounds and not completes the profile of metabolites in the extracts. The findings reveal the presence of various bioactive compounds and validates the earlier reports of therapeutic importance of these plants in the treatment of skin-related diseases. It is strongly recommended that these medicinal plants need further research in the field of natural products to isolate, purify, and characterize bioactive molecules to develop a safety and effective plant-based natural drug for the treatment of various skin ailments.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Joseph B, Priya RM. Bioactive compounds from endophytes and their potential in pharmaceutical effect: A review. Am J Biochem Mol Biol 2011;1:291-309.
2. Dold AP, Cock ML. Preliminary list of Xhosa plant names from Eastern Cape, South Africa. Bothalia 1999;29:267-92.
3. Josia M. Medicinal Properties of Some Plants Used for the Treatment of Skin Disorders in the OR Tambo and Amathole Municipalities of the Eastern Cape Province. Masters Dissertation Department of Botany. Walter Sisulu University; 2013.
4. Moteetee A, Van Wyk BE. The medical ethnobotany of Lesotho: A review. Bothalia 2011;41:209-28.
5. Moteetee A, Kose LS. A review of medicinal plants used by the basotho for treatment of skin disorders: Their phytochemical, antimicrobial and anti-inflammatory potential. Afr J Tradit Complement Altern Med 2017;14:121-37.
6. Pujol J. Natural – The Herbalist Handbook. Durban: Jean Pujol Natural Healer's Foundation; 1990.
7. Indigenous South African Flora. Available from: https://kumbulannursery.co.za/plants/bulbine-asphodeloides. [Last accessed on 2019 Mar 08].
8. Roger O. Harvold Porter Botanical Garden, South African National Biodiversity. 2018. Available from: pza.sanbi.org/helichrysum-petiolare. [Last accessed on 2019 Jan 12].
9. Hutchings A, Scott AH, Lewis G, Cunningham A. Zulu Medicinal Plants. Pietermaritzbg: University Natal Press; 1996.
10. Lourens AC, Reddy D, Başer KH, Viljoen AM, Van Vuuren SF. *In vitro* biological activity and essential oil composition of four indigenous South African *Helichrysum* species. J Ethnopharmacol 2004;95:253-8.
11. Lourens ACU, van Vuuren SF, Viljoen AM, Davids H, van Heerden FR. Antimicrobial activity and *in vitro* cytotoxicity of selected South African *Helichrysum* species. South African J Bot 2011;77:229-35.
12. Sagbo IJ, Mbeng WO. Plants used for cosmetics in the Eastern Cape Province of South Africa: A case study of skin care. Phcog Rev 2018;12:139-56.
13. Ezhilan BP, Neelamegam R. GC-MS analysis of phytocomponents in the ethanol extract of *Polygonum chinense* L. Pharmacognosy Res 2012;4:11-4.
14. Duke J. Phytochemical and ethnobotanical database. Available from: https//phytochem.nal.usda.gov/phytochem/search. [last accessed on 2019 Mar 10].
15. Kontham KV, Leena D, Ganesan S, Sulochana P, Ashok P, Kesanvan MN. 2,4-Di-tert-butyl phenol as the antifungal, antioxidant bioactive purified from a newly isolated Lactococcus sp. Int J Food Microbiol 2015;211:44-50.
16. Durán-Peña MJ, Botubol Ares JM, Hanson JR, Collado IG, Hernandez-Galan R. Biological Activity of Natural Sesquiterpenoids containing a gem-Dimethylcyclopropane Unit. Nat Prod Rep 2015;32:1236-48.

17. Prakasia P, Nair A. Chemical fingerprint of essential oil components from fresh leaves of *Glycosmis pentaphylla* (Retz.) Correa. Pharma Innov J 2015;3:50-6.
18. Karlovic Z, Anic I, Miletic I, Prpic-Mehicic G, Pezelj-Ribaric Sonja, Marsan T. Antibacterial Activity of Halothane, Eucalyptol and Orange Oil. Acta Stomat Croat 2000;22:307-9.
19. Pattnaik S, Subramanyam VR, Bapaji M, Kole CR. Antibacterial and antifungal activity of aromatic constituents of essential oils. Microbios 1997;89:39-46.
20. Jakupovic J, Zdero C, Grenz M, Tsichritas F, Lehmann L, Hashemi-Nejad SM, *et al*. Twenty-one acylphloroglucinol and further constituents from South African Helichrysum species. Photochem 1989;28:1119-31.
21. Lourens AC, Viljoen AM, van Heerden FR. South African *Helichrysum* species: A review of the traditional uses, biological activity and phytochemistry. J Ethnopharmacol 2008;119:630-52.