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Characterization of Essential Oils of *Teclea nobilis* and *Zanthoxylum gilletii*

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Abstract

Essential oils are believed to be rich in bioactive compound which generally contribute to the overall medicinal value of a plant species. Rutaceae plants are mostly used by the traditional herbalist to treat different kind of ailments. Therefore, this research was to determine the occurrence, chemistry, biological activity and possible utilization of *Zanthoxylum giletii* and *Teclea nobilis* as antimicrobial. The extraction of essential oils was conducted in laboratory located in Center for African medicinal flora and fauna, Masinde Muliro University of Science and Technology, Kakamega, Kenya using hydro-distillation in a modified Clevenger apparatus. GC-MS analysis was used to determine the chemical constituent of essential oil of *Zanthoxylum gilletii* and *Teclea nobilis,* (γ-Terpinene, (Z)-3,7-dimethyl-1,3,6-Octatriene and Isoelemicin) and Sabinene, α-Pinene and (R)-4-Methyl-1-(1-methylethyl)-3-cyclohexen-1-ol) were identified as the major composition of *Teclea nobilis* and *Zanthoxylum giletii* respectively. γ-Terpinene (0.64-3.76%), α-Humulene (0.08-1.58%), α-Pinene (1.38-26.20%), α-Cubebene (0.03-0.6%) and Caryophyllene oxide (0.24-7.09%) were identified in all the essential oils of plants used in current study. The presence of oxygenated monoterpenes and sesquiterpenes suggests that the essential oils could be effective in managing secondary infections.

Keywords: Essential oils, *Teclea nobilis, Zanthoxylum gilletii,* bioactive compound, Rutaceae, antimicrobial

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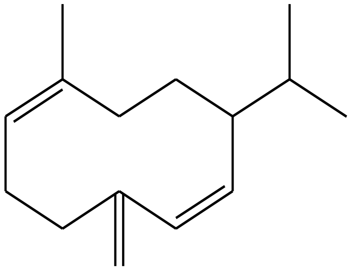
Introduction

Rutaceae are commonly known for their flowering nature, they are widely commonly known as citrus family which is in order sapindales (Groppo et al., 2022). Bayer *et al.,* 2009 reports that family consist of about 158 genera and 1900 species that are of diverse morphological characteristics. Rutaceae plants have economical value in that they are being used as; source of food, essential oils, medicinal herbs and pharmaceuticals (Ling *et al*., 2009). These plants are distributed across the world but mainly in tropical and temperate regions (Groppo et al., 2008). The plants in this family have been described to have essential oils.

Most species from this family have been used to control insects for instance *Clausena anisata* has been used treat of myiasis through expulsions of maggots from animal skins (Chavunduka 1976). In addition, from the recent study it has described rutaceae family to contain antibacterial activities (Bibi *et al.,* 2024).

Predio *et al.,* 2018 describes essential oils to show the following characteristics; antiviral, anti-parasitic, ant-mycotic, antioxygenic, insecticidal and antimicrobial properties. The aroma of plants and their essential oils are the primary sources of making of some compounds for different applications (Sinha *et al*., 2024). Essential oil has bioactive compounds which could be used in treatment of secondary infections. This could attribute for these essential oils in treatment of secondary infections (Omorodion & Adetunji, 2024). Rutaceae plant contains appreciated amount of essential oil which has high percentage of monoterpenes and sesquiterpenes (Japhet *et al*., 2014). *Teclea nobilis* and *Zanthoxylum gilletii* essential oils were investigated for their chemical profile which has contributed to their medicinal value especially for the treatment of secondary infections caused by tungiasis.

Teclea nobilis belong to the genus Teclea which has been subsumed into *Vepris* due to their morphological characteristics (Morton 2017). It is locally known as Kuriot by the Kipsigis community. *Teclea nobilis* are smooth evergreen shrub that grows 5-12m high but grows beyond that in rainy forest. The fruits are used as source of food (Abuka *et al.,* 2024) and leaves are used by the Kipsigis community in treating chronic coughs especially on young children the same way it has been used in Ethiopia for the same remedy (Kisangau *et al.,* 2007; Omujal *et al.,* 2020). The leaves have been found to show antipyretic, analgesic, inflammatory and antimicrobial activities (Adedokun 2023; Tegegne, 2023). The stem barks are used to treat gonorrhea (Bussmann *et al.,* 2020; Ombito *et al.,* 2021). It is documented that it has strong anti-inflammatory activity that is comparable to that of diclofenac (Omujal *et al*., 2020). The sesquiterpenes that has anti-inflammatory effect namely: Germacrene-D [**1**] (Barrero *et al*., 2008); α-cubebene [**2**] (Choi *et al*., 2009); α-Cadinol [**3**] (Tung *et al.,* 2010).



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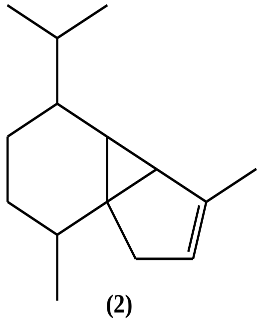

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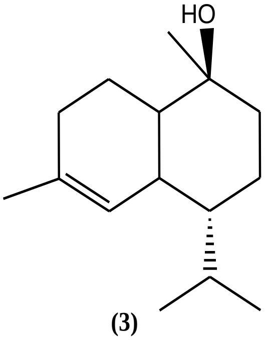

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It is reported that it can be used in controlling of insects as it has insect antifeedant and antibacterial activities (Fenibo *et al.,* 2023; Ayele *et al.,* 2023). This species has been found to have essential oils from the leaves (Al-Rahaily, 2001) and the roots (Ocheng *et al.,* 2015). This study was carried to determine the chemical profile of the essential of this species and comparison with other studies on the same. The chemical profile of essential oil of *T. nobilis* leaves are illustrated in the Table 1.

Table 1: Chemical profile of EO of *T. nobilis* leaves

Plant species	Location	Major compounds
T. nobilis	Kenya	β- ocimene (10.15 %), γ- terpinene (6.11 %), α- pinene (3.95 %), neoallo-ocimene (3.68 %) and limonene (3.34 %), β- cadinene (4.98 %), 1,6- germacradien-5-ol (4.38 %), α-amorphine (3.96 %), τ-cadinol (3.56 %) and germacrene D (3.06 %) (Njogu *et al.,* 2014)
	Saudi Arabia	Germacrene-D (19%), Ocimene isomers (22.3%), Guaiol (3.9%), Elemol (2.9%) and Bulnesol (2.5%) (Al-Rehaily, 2001)
	Uganda	Trans-β-ocimene (8.5%), δ-cadinene (7.3%), γ-elemene (2.4%), Germacrene-D (54.4%), α-Gurjunene (4.9%), α-cadinol (9.1%), τ-cadinol (2%), Nerolidol (1.9%), Methyl isoeugenol (1.7%) and palmitic acid (2.1%) (Ocheng *et al*., 2015)

Z. gilletii is an evergreen, aromatic deciduous shrub or tree that belongs to the family rutaceae (Negi *et al.,* 2011). It is a tropical rainforest species, distributed between altitudes ranging from 900 to 2400m. It is locally known as Sakawaita by the Kipsigis community. This species grows from 10 - 35 metres tall though in some areas it can be as small as 4 metres or up to 40 metres. The straight, cylindrical bole is normally free of branches for up to 15 metres or but in some cases up to 25 metres. The bole is usually 10 - 30cm in diameter, but can be up to 150cm often with indistinct buttresses at the base. It has strong and straight corky thorns which measures 1 - 3cm long and they are found on the bole and branches (Khalil *et al., 2024*).

Z. gilletii has added value to the people for its economic and medicinal importance (Kathambi *et al.,* 2020). The

Luhya community; that is, a major habitat of this region uses the bark of *Z. gilletii* in traditional anti-malaria preparations (Nyunja *et al.,* 2009). Its essential oils have been studied on the effectiveness over the control of mosquitos and it was found to have larvicidal activity (Japeth *et al*., 2014). The major compounds that have been identified in *Z. gilletii* is as shown in Table 2.

Table 2: Chemical profile of essential oils of *Z. gilletii's* leaves

Plant Species	Location	Major Compounds
Z. gilletii	Kakamega, Kenya	γ-terpinene (10.62%), β-myrcene (5.16%), sabinene (4.89%), β-ocimene (3.12%), camphene (2.56%) trans-caryophyllene (9.82%), caryophyllene oxide (4.4%), α-cadinol (2.71%), 1, 1, 4, 8-tetramethyl-4, 7, 10- cycloundecatriene (2.62%), δ-cadinene (2.52%) and τ-cadinol (2.29%) (Japhet *et al*., 2014).

Materials and methods

Collection and identification of plant materials

The leaves of *Teclea nobilis* were collected from their natural habitat at Sossiani River, Uasin Gishu County (0.504477° N and 35.288308° E) with the assistance of Mr. Wanjohi (taxonomist) from University of Eldoret and leaves of *Zanthoxylum gilletii* were collected in their natural habitat in Kakamega forest, Kakamega County. All the plants' leaves were collected in June 2020. The plants were identified by Mr. Wanjohi (taxonomist) and voucher specimens kept at Herbarium, University of Eldoret.

Experiment site

The essential oils extraction was carried out in Masinde Muliro University of Science and Technology (MMUST) in the laboratory located at the Center for African Medicinal and Nutritional Flora and Fauna (CAMNFF), Kakamega County, Kenya.

Extraction of essential oils from the selected species

Fresh leaves (300g) of each species were put in 5-litres double-necked round bottom flask. Distilled water was added until it covers the plant materials (300ml) and then it was subjected to hydro-distillation for 3 hours. The essential oils distillate was dried over anhydrous sodium sulphate, packed in sealed glass vials and kept at 4°C (Stashenko *et al*., 2004 and Lucchesi *et al*., 2004).

Analysis of the essential oil constituents of the selected plant species

Analyses of the oils and identification of the components were carried out by GC, GC-MS and GC co-injection of the essential oils with authentic samples. Analyses were performed on a capillary gas chromatograph, Hewlett Packard (HP) 5890 Series II, equipped with a split-less capillary injector system, 50 m x 0.2 mm (i.d) cross-linked methyl silicone (0.33 film thickness) capillary column and FID coupled to HP 3393A Series II integrator. The carrier gas was N_2 at 280°C at 5°C/Min and a hold at this temperature for 10 min. GC-MS analyses were carried out on a HP 8060 Series II Gas chromatograph coupled to a VG platform II Mass Spectrometer. The MS was operated in the EI mode at 70 eV and an emission current of 200 A. The temperature of the source was held at 180°C and the multiplier voltage at 300V. The pressure of the ion source and MS detector were held at 9.4 x 10^{-6} and 1.4 x 10^{-5} bar respectively. The MS had a scan scale cycle of 1.5 s (scan duration of 1 s and inter-scan delay of 0.5 s). The instrument was calibrated using heptacosafluorotributyl amine, $[CF_3(CF_2)_3N$, (Apollo Scientific Ltd., UK). The column used for GC-MS was the same as the one described for GC analysis except for the film thickness. The temperature programme involved an initial temperature of 50°C (5 min), to 90°C at 5°C min^{-1}, to 200°C at 2°C min^{-1}, to 280°C at 20°C min^{-1} and a hold at this temperature for 20 min. Identification of the components was made by comparison of mass spectra with published data (NIST, Wiley libraries) and confirmed, where possible by GC co-injection with authentic samples (Adams, 2001; Yang et al., 2011).

Results and discussion

Considering only constituents present in >1%, γ-Terpinene (0.64-3.76%), α-Humulene (0.08-1.58%), α-Pinene (1.38-26.20%), α-Cubebene (0.03-0.6%) and Caryophyllene oxide (0.24-7.09%) were identified in all the essentials oils.

Teclea nobilis

A total of 105 compounds were identified in *Teclea nobilis* fresh leaf oil (Table 3: Figure 1) which accounts for 90% of the total leaf oil composition of the plant as identified by GC-MS and GC-coinjection with authentic standards, monoterpene hydrocarbons were found in higher amounts. Thirty compounds were found to have constituents >1%.

Table 3: Major chemical constituent of *T. nobilis* species' essential oils

GC-peak	RI	Compound identified	Chemical formula	% Area	Area
17	9.82	α-Pinene	$C_{10}H_{16}$	1.38	2.82E+08
21	10.74	γ-Terpinene	$C_{10}H_{16}$	15.07	3.09E+09
22	11.05	β-Pinene	$C_{10}H_{16}$	3.83	7.85E+08
25	11.51	δ -2-Carene	$C_{15}H_{24}$	0.50	1.02E+08
27	11.76	Limonene	$C_{10}H_{16}$	4.32	8.86E+08
28	11.96	(Z)-β-Ocimene	$C_{10}H_{16}$	8.54	1.75E+09
29	12.17	(Z)- 3,7-dimethyl-1,3,6-Octatriene	$C_{10}H_{16}$	14.93	3.06E+09
34	12.80	2-Carene	$C_{10}H_{16}$	2.17	4.45E+08
35	12.99	3,7-dimethyl-1,6-Octadien-3-ol	$C_{10}H_{18}O$	1.43	2.93E+08
38	13.47	allo-Ocimene	$C_{10}H_{16}$	2.18	4.47E+08
42	14.30	(R)-4-methyl-1-(1-methylethyl) -3-Cyclohexen-1-ol	$C_{12}H_{20}O_2$	2.42	4.97E+08
43	14.48	L-α-Terpineol	$C_{10}H_{18}O$	1.18	2.41E+08
51	15.76	(Z,Z)-1,3-Cyclooctadiene	C_8H_{12}	0.97	1.99E+08
52	15.90	Z-Anethole	$C_{10}H_{12}O$	2.14	4.38E+08
53	16.17	Methyl 4,7,10,13-hexadecatetraenoate	$C_{17}H_{26}O_2$	1.00	2.04E+08
63	17.46	Methyl eugenol	$C_{11}H_{14}O_2$	1.67	3.43E+08
64	17.59	Methyl N-Methyl anthranilate	$C_8H_9NO_2$	0.94	1.92E+08

73	18.56	Germacrene D	$C_{15}H_{24}$	1.20	2.45E+08
74	18.68	Z-Methyl isoeugenol	$C_{11}H_{14}O_2$	3.55	7.27E+08
75	18.77	α-Farnesene	$C_{15}H_{24}$	2.18	4.46E+08
77	19.04	1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)- Naphthalene	$C_{15}H_{24}$	0.98	2E+08
80	19.35	Elemicin	$C_{12}H_{16}O_3$	1.74	3.57E+08
82	19.62	(E)-2-Butenoic acid, 2-(methylenecyclopropyl)prop-2-yl ester	$C_{11}H_{16}O_2$	1.36	2.79E+08
83	19.72	Germacra-1,6-dien-5-ol	$C_{15}H_{26}O$	0.79	1.62E+08
85	20.02	β-Elemenone	$C_{15}H_{22}O$	0.60	1.23E+08
87	20.54	Isoelemicin	$C_{12}H_{16}O_3$	12.10	2.48E+09
88	20.64	γ-Muurolene	$C_{15}H_{24}$	1.24	2.54E+08
90	20.90	1,2,3,4,5,6-hexahydro-1,1,5,5-tetramethyl-, (2s-cis)-2,4a-Methanonaphthalen-7(4aH)-one,	$C_{15}H_{22}O$	0.64	1.31E+08
91	21.14	Germacrone	$C_{15}H_{22}O$	1.31	2.69E+08

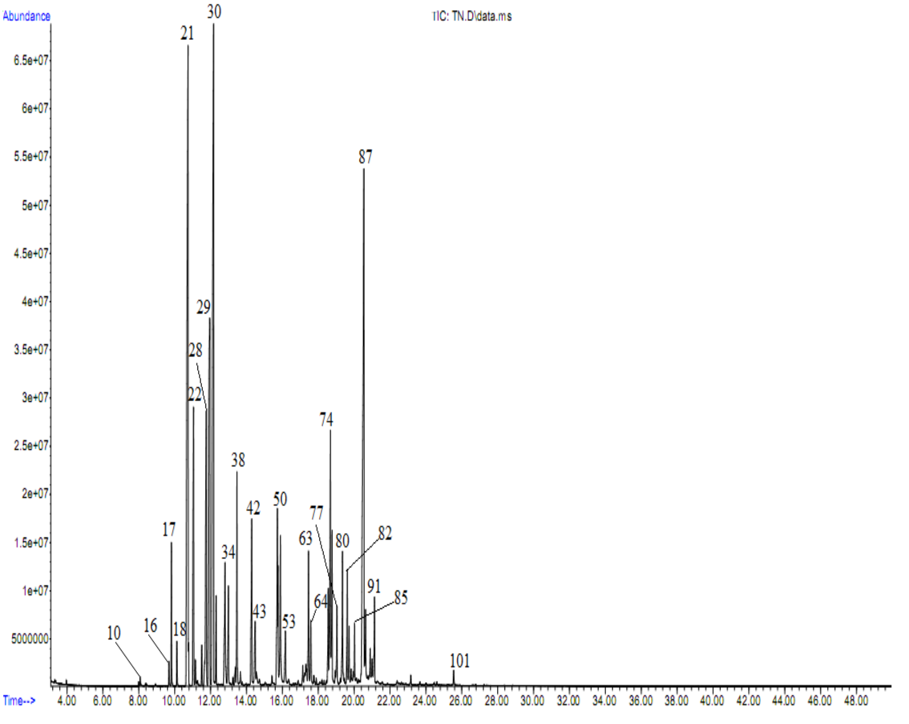

Figure 1: Total ion chromatogram of essential oils of *T. nobilis*

Monoterpene hydrocarbons were found to be present in higher amounts. Thirty compounds were identified to have constituents exceeding 1%, showcasing their significance in the overall chemical profile of *Teclea nobilis* fresh leaf oil. The monoterpenes identified included α-Pinene, Limonene, β-Pinene, 2-Carene, Z)-β-Ocimene, allo-Ocimene, γ-Terpinene, and L-α-Terpineol. In addition, the most dominant sesquiterpene hydrocarbons identified in the essential oil of *Teclea nobilis* included Germacrene D, α-Farnesene, Elemicin, γ-Muurolene, and Germacrone. Sesquiterpenes play a crucial role in the overall scent and potential medicinal properties of essential oils. Monoterpenes such as α-Pinene, Limonene, β-Pinene, and γ-Terpinene, identified in *T. nobilis*, have been reported as common constituents in essential oils with diverse biological activities (Jemâa *et al*., 2012; Sharmeen, *et al*., 2021).

Understanding the major constituents and their abundance contributes to the knowledge of the plant's essential oil profile and potential applications in various fields such as in pesticide (Jemâa *et al*.,2012), aromatherapy, traditional medicine (Matasyoh, 2020) and the fragrance industry (Sharmeen *et al*., 2021). These monoterpenes are known for their diverse biological activities and contribute to the aroma and potential therapeutic properties of the essential oil (Al-Rehaily, 2001; Omujal, 2021).

Zanthoxylum gilletii

The GC-MS chromatogram of the essential oil of the fresh leaves of the plant is presented in Figure. From the analysis, a total of 66 compounds were identified from the essential oils of *Zanthoxylum gilletii* accounting for 82%, monoterpene hydrocarbons were found in higher amounts. Considering only constituents present in >1% eleven compounds were identified as illustrated in Table 4.

Table 4: Major chemical constituent of *Z. gilletii* essential oils

GC-Peak	RI	Compound identified	Chemical Formula	% area	Area
9	9.8261	α-Pinene	$C_{10}H_{16}$	26.20	4.27E + 08
10	10.1186	Camphene	$C_{10}H_{16}$	5.68	92538630
11	10.6685	Sabinene	$C_{10}H_{16}$	27.98	4.56E + 08
12	10.7153	β-Pinene	$C_{10}H_{16}$	6.20	1.01 + 08
13	11.0195	β-Myrecene	$C_{10}H_{16}$	6.56	1.07 + 08
17	11.6513	*o*-Cymene	$C_{10}H_{14}$	6.99	1.14E + 08
18	11.7332	β-Phellandrene	$C_{10}H_{16}$	9.75	1.59E + 08
20	12.0842	*E*- β-Ocimene	$C_{10}H_{16}$	14.48	2.36E + 08
21	12.2772	γ-Terpinene	$C_{10}H_{16}$	3.76	61349882
24	12.9851	Linalool	$C_{10}H_{18}O$	9.20	1.5E + 08
30	14.2779	(R)-4-Methyl-1-(1-methylethyl)-3-cyclohexen-1-ol	$C_{12}H_{20}O_2$	21.54	3.51E + 08

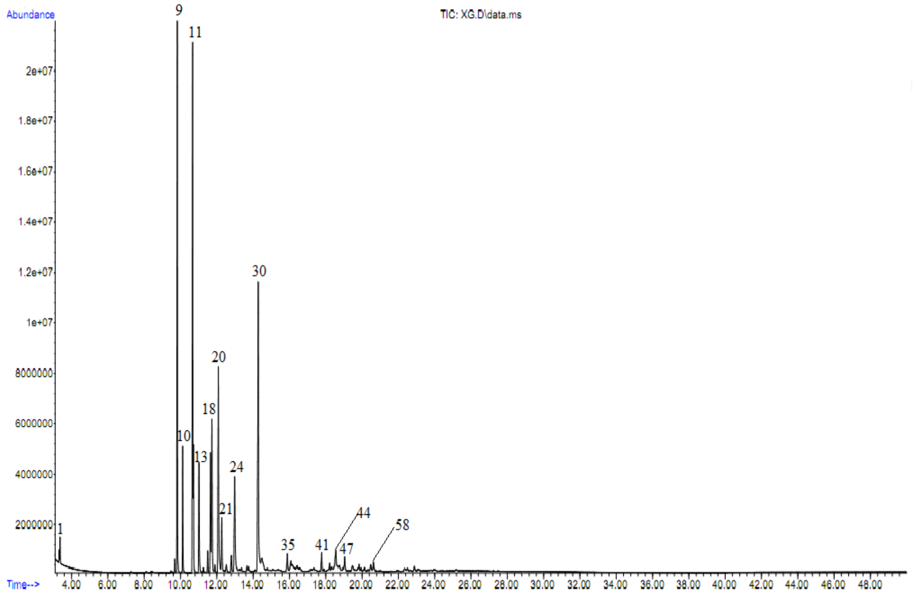

Figure 2: Total ion chromatogram of essential oils of *Z. gilletii*

Z. gilletii essential reported major monoterpenes as Sabinene and α-Pinene. The dominance of monoterpene hydrocarbons in *Z. gilletii* fresh leaf oil aligns with the findings of previous studies on essential oils from various plant sources (Ombito *et al*., 2014). Monoterpenes such as α-Pinene, Limonene, β-Pinene, and γ-Terpinene, identified in *Z. gilletii*, have been reported as common constituents in essential oils with diverse biological activities. The findings are in line with those of Japeth *et al*. (2014) that the larvicidal activity of *Z. gilletii* is attributed to the presence of γ-terpinene, β-myrcene, sabinene, trans-caryophyllene, caryophyllene oxide, α- cadinol and germacrene D which have been associated with larvicidal activity against different species of mosquito.

The difference was also noted in the composition of sesquiterpenes where Germacrene D was the major compound whereas Caryophyllene was the major sesquiterpene. This can be alluded to factors such as, geographical situation, extraction method, growth conditions, environmental factors and genetics. Phytochemical investigations of other members of *Zanthoxylum* species have revealed the presence of alkaloids of various skeletal types, lignans, coumarins amides as common secondary metabolites which also have chemotaxonomic importance to the genus. Other metabolites such as flavonoids and sterols have also been isolated from plants from this genus (Waterman and Grundon, 1983; Adesina, 2005).

Essential oils have various physiological effects on humans and other mammalian species when inhaled or ingested and have been utilized in aromatherapy for treatment of different ailments. Plants oils from *Zanthoxylum* genus have been investigated and reported for various biological activities. These activities include insecticidal, fungicidal, antibacterial, and fumigant activities (Prieto *et al*., 2011; Christofoli *et al*., 2015). Essential oils from *Zanthoxylum giletii* have been reported to possess larvicidal activity against *Anopheles gambiae* (Ombito *et al.*, 2014).

Moreover, the presence of sesquiterpene hydrocarbons, including Germacrene D, α-Farnesene, Elemicin, γ-Muurolene, and Germacrone, further contributes to the complexity and potential therapeutic properties of *Z. gilletii* essential oil. These sesquiterpenes have been identified in various essential oils and have been associated with different biological activities (Rosato *et al*., 2018; Sadgrove, 2022).

Conclusion and recommendation

The plants used in the current study though they belong to the same family but their chemical profile much differ from each other as witnessed in the essential oils. The morphology of the plant could attribute to its chemical profile. Plants are rich source of bioactive organic chemicals and offer an advantage on treatment of different ailment over the use of chemicals. The essential oils are naturally non-toxic and therefore, could be used in treatment and management of secondary infections caused by tungiasis. The chemical profile of *Z. gilletii* and *T. nobilis* showed rich in monoterpenes and Sesquiterpenes and especially oxygenated constituent which have been found to have antibacterial activities. This finding offers alternative way of treating secondary infections of *Tunga penetrants* in western Kenya.

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