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BIOACTIVE COMPOUNDS OF *PILIOSTIGMA THONNINGII* ESSENTIAL OIL DETECTED BY THE GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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ABSTRACT

This study aimed to investigate the bioactive compounds present in *Piliostigma thonningii* essential oil (PEO) using the gas chromatography. Essential oil was extracted using the steam distillation method. . A total of about 32 active compound were detected including their percentage area and mass peaks. The active compounds include; Azulene, beta myrcene, beta limonene, cis- linaloxide, alpha cymene, beta ocimene, trans beta ocimene, 4-methylpropiohenone, 1,3 dimethyl-4-Isoprophylbenzene, dodecane, tridecane, 2,3-dimethylnephthalene, 1,8-dimethylnepthalene, alpha thujene, beta pinene, terpinen-4-ol, alpha terpineol, gamma terpineol, alpha cubebene, alpha yiangene, alpha selinene, chrysathenine, alpha allyltoluene, borneol, hexenyl acetate, camhene, n-nonanol, trans-pinocarveol, cis pinocarveol, Fenchol and alpha muurolene. However, the major bioactive compound are beta pinene (25.02%), beta limonene (12.85%) and alpha muurolene (9.63%).

1. Introduction

Essential oils or volatile oils are mainly aromatic oily liquids extracted by distillation from plant parts, such as flowers, buds, seeds, leaves, twigs, bark, wood, fruits and roots (Miguel, 2010). They are steam-volatile or rather organic-solvent extracts which have so far been used traditionally for many centuries in various parts of the world. They are basically known to have pleasant flavour and aroma, as well as preservative properties. EOs usually contain a rich amount of different compounds, such as terpenes, alcohols, acetones, phenols, acids, aldehydes and esters (Negi, 2012). These substances can exert defensive roles against bacterial, fungal or insect attacks. Studies on EOs have reported several positive effects on feed utilisation, animal health and live performance of rabbits (Celia *et al*., 2016). Camel's foot (*Piliostigma thonningii* Schum.) Milne – Rech (Caesalpiniaceae) is a small tree often found in the savannah, which is of crooked growth with dark brown to black fissured bark. *Piliostigma thonningii* has been used for many years in treatment of dermatosis and malaria by Nigerian traditionalists and is currently shown to possess typical flavouring, anti-oxidant, insecticidal and antimicrobial properties (Tira-Picos *et al*., 2010).

Piliostigma thonningii is a leguminous plant belonging to the family *Leguminosae -Caesalpinioideae*, a family that comprises of trees, shrubs or very rarely scramblers. The tree is perennial in nature and its petals are white to pinkish colour produced between November and April. The fruit is hairy, hard and flattish pod, which turns rusty brown, woody, twisted and splits at ripening and usually persistent on the tree between June and September (Jimoh and Oladiji, 2005). The plant usually grows as small to medium-size tree to 8m high short twisted bole and twisted branches. The wood is reddish-brown turning dirty brown (Burkill 1995).

The leaves are edible and chewed to relieve thirst by the Masai of East Africa. The fruit and seeds are also edible. The pods and foliage are nutritious and relished by cattle and elephant (Burkill 1995). The inner bark is used to make rope.

Locally, the seed is called Abefe in the Yoruba land (Nigeria). Other names include Monkey bread, Camel's foot, Kalgo (Hausa) and Okpoatu (Ibo). *P. thonningii* grows in open woodland and savannah regions that are moist and wooded grassland in low to medium altitudes. It is widely distributed in Africa and Asia. It is found growing abundantly as a wild uncultivated tree in many parts of Nigeria such as Zaria, Bauchi, Ilorin, Plateau, Lagos and Abeokuta (Schultes and Hofmann, 1973; Djuma, 2003). The seeds of *P. thonningii* fruits have been reported to be eaten by African antelope and elephant while farmers in the lower Savanna region grind up the seed as fodder for cattle during winter months (Djuma, 2003).

A general review given below aims to deal with the special topic of EOs use in various animal species nutrition. Therefore, it includes examples from research studies which examine the various modes of action of these natural substances. In recent years, products containing EOs have been used as feed additives to promote the performance of productive animals, and also as food additives. These products could have different modes of action, including enhanced feed consumption and flavour, stimulation of the digestive enzymes' secretion, increased gastric and intestinal mobility, antimicrobial, antiviral, antiparasitic, immuno-mudulating, antioxidant and anti-inflammatory activities and lastly as a preservative mechanism in foods.

Although some EOs have been evaluated in livestock diets with positive results, nothing or little is known about the effect of Camel's foot (*Piliostigma thonningii* Schum) and its bioactive compounds.

2. Methodology

Collection of P. thonningii seed and the extraction of its essential oil

Piliostigma thonningii seeds was sourced from within the Guinea Savannah agro ecological zone. It was identified and authenticated by a certified taxonomist at the Department of Biological Science, Forestry Research Institute of Nigeria (FRIN). The *P. thonningii* seeds were shade-dried, finely ground and stored at room temperature until extraction. Steam distillation was the method of extraction used. The essential oil was extracted with a Clevenger apparatus using the method described by Mohamed *et al*. (2006); About 100 g of dried ground sample was suspended in 700 ml of distilled water, where steam distillation process was employed at 100 ^{o}C for about 3 hours by placing in steel apparatus. The setup was by heated up after connecting the condenser to a water inlet and outlet. This process allowed for softening of the sample and letting (discharging) the end product essential oil in vapourised form. The vapourized essential oil droplets formed and mixed with the steam (the carrier) and subsequently converged into a cooling system. Following convergence in the cooling system, the essential oil was then collected via a collection tube. The percentage of the oil content was calculated using the formulae below:

% Oil content (v/w) = volume of the oil extracted (ml) / weight of the sample taken (g) x 100%

Determination of the bioactive constituents of P. thonningii essential oil using gas chromatography–mass spectrometry (GC-MS)

GC-MS analysis of the oils was carried out in a Shimadzu GC-MS-QP-2010 plus chromatography with capillary column (methyl phenyl siloxane, 60 mm x 0.25 mm). The instrument operating conditions were: Carrier gas will be helium with flow rate 1.6 mL/min. Column oven temperatures at 4 min and 8 min will be 60^{0}C and 160-280^{0}C respectively. Injection temperature was set at 250^{0}C; volume was 8µL in split ratio1:0. The MS temperature source was set at 200^{0}C with interface temperature of 250^{0}C and solvent cut time of 2.5 min. The ionization mode was electron ionization and the mass range of m/z 40 to 400 while scan speed was 769 amu/s, start time and end time was about 3.0 min and 27 min. respectively.

Identification of chemical constituents

Identification of individual chemical constituents was based on the molecular formulae, molecular mass, retention index and percentage concentration (peak area). Interpretation of spectrum was conducted using the database of National Institute Standard and Technology (NIST 05). The name, molecular weight and structure of the components of the oils were ascertained.

4. Results and Discussion

Bioactive compounds of *P. thonningii* essential oil detected by the gas chromatography-mass spectrometry

A total of about 32 active compound were detected including their percentage area and mass peaks. The active compounds include; Azulene, beta myrcene, beta limonene, cis- linaloxide, alpha cymene, beta ocimene, trans beta ocimene, 4-methylpropiohenone, 1,3 dimethyl-4-Isoprophylbenzene, dodecane, tridecane, 2,3-dimethylnephthalene, 1,8-dimethylnepthalene, alpha thujene, beta pinene, terpinen-4-ol, alpha terpineol, gamma terpineol, alpha cubebene, alpha yiangene, alpha selinene, chrysathenine, alpha allyltoluene, borneol, hexenyl acetate, camhene, n-nonanol, trans-pinocarveol, cis pinocarveol, Fenchol and alpha muurolene. However, the major bioactive compound are beta pinene (25.02%), beta limonene (12.85%) and alpha muurolene (9.63%).

Table 1: Bioactive compounds detected by the gas chromatography-mass spectrometry

Compounds	Percentage area	Mass peak
Azulene	0.42	25, 40, 42, 57
Beta myrcene	6.05	25, 72, 102, 106, 110
Beta limonene	17.85	25, 41, 43, 57
Cis- linaloxide	0.21	25, 39, 47, 49, 55
Alpha cymene	0.15	25, 44, 48
Beta ocimene	0.06	25, 88, 106
Trans beta ocimene	0.30	25, 39, 66, 70
4-methylpropiohenone	0.12	25, 88, 106, 109
1,3 dimethyl-4-Isoprophylbenzene	3.11	25, 48, 51, 58, 69
Dodecane	0.19	25, 41, 43
Tridecane	1.20	60, 68, 77, 103
2,3-dimethylnephthalene	0.71	25, 44, 68, 92
1,8-dimethylnephthalene	0.22	25, 81, 87, 102, 117
Alpha thujene	0.19	25, 29, 38, 44

Beta pinene	25.02	25, 62, 68, 100
Terpinen-4-ol	2.60	25, 42, 47, 58
Alpha terpineol	3.70	25, 88, 93, 106
Gamma terpineol	5.11	25, 71, 79, 100
Alpha cubebene	2.09	25, 63, 69
Alpha yiangene	1.02	25, 41, 55, 108, 127
Alpha selinene	1.10	25, 40, 48, 51
Chrysathenine	0.08	25, 47, 57,63
Alpha allyltoluene	0.29	25, 39, 47, 58
Borneol	1.44	25, 33, 39
Hexenyl acetate	2.83	25, 28, 37, 108
Camhene	1.50	25, 63, 68, 101
n-Nonanol	0.04	25, 69, 104
Trans-pinocarveol	0.62	25, 27, 29, 41, 55, 67
Cis pinocarveol	1.77	25, 41, 55, 67, 81, 104
Fenchol	0.96	25, 41, 58, 138
Alpha muurolene	9.63	25, 39, 51, 105, 109

DISCUSSION

Thirty two bioactive compounds including azulene, beta myrcene, beta limonene, cis- linaloxide, alpha cymene, beta ocimene, trans beta ocimene, 4-methylpropiohenone, 1,3 dimethyl-4-Isoprophylbenzene, dodecane, tridecane, 2,3-dimethylnephthalene, 1,8-dimethylnepthalene, alpha thujene, beta pinene, terpinen-4-ol, alpha terpineol, gamma terpineol, alpha cubebene, alpha yiangene, alpha selinene, chrysathenine, alpha allyltoluene, borneol, hexenyl acetate, camhene, n-nonanol, trans-pinocarveol, cis pinocarveol, fenchol and alpha muurolene were detected and identified. This was similar to Ajiboye *et al*. (2017) who detected and identified 29 compounds in PTO, and further reported that 9-octadecenoic acid (oleic acid) was dominated in PTO by (15.4%). This slight difference in amount of bioactive compounds detected may have been due to the method of the oil extraction process employed. However, steam distillation process as described by Mohamed *et al*. (2006) was employed in the current study which involved the use of a Clevenger apparatus due to the highly volatile nature of the EO.

Beta pinene was the most abundant bioactive compound in PEO. β-pinene are well-known representatives of the monoterpenes group, and are found in many plants' essential oils. A wide range of pharmacological activities have been reported, including antibiotic resistance modulation, anticoagulant, antitumor, antimicrobial, antimalarial, antioxidant, anti-inflammatory, anti-Leishmania, and analgesic effects, for β-pinene (Bahare *et al*. 2019). The pinene also posseses anaesthetic properties and acts as an acetylcholinesterase inhibitor, blocking the normal breakdown of acetylcholine in the body system. They are majorly a monoterpene hydrocarbon giving PEO its characteristic green colour. Similarly, the β limonene and β-mycerne function as a flavouring agent masking the bitter taste of the EO and possess antimicrobial properties (NCBI, 2017). Pandi *et al*. (2021) reported limonene as monoterpenes which possesses antioxidant, antidiabetic, anticancer, anti-inflammatory, cardioprotective, gastroprotective, hepatoprotective, immune modulatory, anti-fibrotic, anti-genotoxic. According to Inui *et al*. (2013), β-mycerne has a characteristic aroma improving functions making the EO readily accepted by consumer. myrcene is generally known to also possess anti-inflammatory effect, anti-tumor, sedative, and other health benefits to animals. β-myrcene enters into plasma unaltered, with a peak concentration between 2 to 4 hours, explaining its pharmacokinetics in animal metabolism (Papada *et al*., 2020). β-myrcene may generate synergistic interactions hiking up their potential benefits in the animal (Sterba *et al*., 2015)

Gamma terpineol, hexenyl acetate, fenchol and camhene are bioactive compounds present in EO (Yuasa and Yuasa, 2006). They have functions similar to the flavouring and aroma characteristic of the β-pinene (Ethan and Jahan, 2017). Hexenyl acetate possesses a characteristic fruity odour (Gomez and Ledbetter 1994).

Similarly, n-nonanol has relative flavouring characteristic but can be harmful to internal body organs when large doses are administered and ingested (HSDB, 2006).

Trans-pinocarveol and Cis- pinocarveol are monoterpene hydrocarbons having sedative characteristics. They are GABA(A) modulators producing the muscle relaxant effect. The 4-methylpropiohenone bioactive compound similarly have anaestheic and muscle relaxant property but is harmful when ingested in large quantity (HSDB, 2006).

The alpha cymene, Beta ocimene and Trans beta ocimene are monoterpenes hydrocarbon and constituents of EO having antifungal properties and improves odour of the oil mixture (SCLabs, 2016). The major bitter taste of EO is due to the bioactive compound Alpha thujene, which is a monoterpene having insecticidal properties with no much significance to health (SCLabs, 2016).

Azulene, 1,3 dimethyl-4-isoprophylbenzene, dodecane, tridecane, 2,3-dimethylnephthalene and 1,8- dimethylnaphthalene are bioactive compounds majorly obtained via steam distillation method of EO extraction and are harmful in large quantity (Churchill, 2007).

The alpha cubebene, alpha yiangene, alpha selinene, chrysanthenine, alpha allytoluene and alpha muurolene are generally susquiterpene hydrocarbons exhibiting antifungal, antioxidant and antiinsecticidal and may be harmful in large quantity (SCLabs, 2016). These bioactive compound confer the antimicrobial, antioxidant, anti-inflammatory, anti-helminthic, anti-lipidermic properties.

It is worthwhile to indicate that there are no data in the literature of the chemical and physical properties of *P. thonningii* seed oil for comparison.

5. Conclusion

The findings presented showed that the extraction of PEO using the steam distillation method and determination of bioactive compounds detected by the gas chromatography-mass spectrometry and subsequent identification of bioactive compounds showed about 32 compounds which had a higher number of nontoxic components possessing several properties including the; antioxidant, antimicrobial, antibiotic, antifungal, acetyl cholinesterase inhibitor, anti-inflammatory, anti-helminthic, anti-lipidermic etc. implying no threat in domestic animals and may be administered to or supplemented in the diet of domestic animals. It may also be used as a replacement to antibiotics.

Conflicts of Interest

The author declares no conflict of interest in the design, collection, writing of manuscript and decision to publish this work.

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