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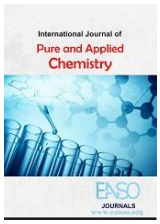

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Original Article

Gas Chromatography-Mass Spectrometry Analysis of Hydrodistilled Essentials Oils from *Solanecio manii*

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Solanecio Manii, Crassocephalum Manii, 3-Carene, Gas Chromatography-Mass Spectrometry, Hydrodistillation, Essential Oils.

ABSTRACT

The leaves of Solanecio manii were collected from sampled plants in Kandara Sub-County Muranga County in Kenya. The aim of collection of the leaves was to carry out the extraction and analysis of essential oils from S. manii. The leaves were transported to the pharmacognosy laboratory at the Mount Kenya University. The leaves were washed with running tap water and rinsed with distilled water to remove physical and chemical contaminants. They were air dried on the laboratory benches for seven days to lose 90% of moisture content. The essential oils (Eos) were extracted from 200.000 g of dry leaves by hydrodistillation using the Clavenger apparatus for 8 hours. The rate of essential oil production from the leaves was computed and found to be 0.01%. GC-MS instrument was utilized to analyse the qualitative and quantitative composition of essential oils. The compounds that were found present in the total ion chromatogram were, 3-Carene (40%), Limonene (35.48%), 1-undecanol (8.0%), β-pinene (6.4%), Menthol (1.6%), β-Cymene (1.1%) Longifolene (0.78%) and α-terpenolene (0.5%).

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INTRODUCTION

Essential oils are plant-derived volatile and aromatic chemicals (Krishnaveni & Santhoshkumar, 2017). They can generally be categorized as secondary metabolites despite the fact that they may belong to other distinct classes of natural chemicals (Pourian et al., 2009). Essential oils serve as attractants and protectants in plants which are ecological roles that increase survival different from basic metabolic processes like growth and reproduction (Aziz et al., 2018). Essential oils have been used for a variety of purposes, including pharmacology, cosmetics, food, hygiene, and perfumery in raw or refined form or to create chemotypes. Over 3000 essential oils have been studied to date (Aziz et al., 2018). Some of the essential oils that have wide applications are derived from lime, bergamot, tangerine, lemon, sweet orange, mandarin, Cinnamon, Citronella, lemongrass, petitgrain, lavender, palmarosa, patchouli, Geranium, rosemary, spike lavender, Ginger, vetiver, neroli (orange blossom), rose, Jasmine, and ylang-ylang plants (Naeem et al., 2018). From previous studies, a small percentage of plant essential oils have been put into commercial use. Therefore, essential oil research and its prospective uses as a subject have not been fully studied, and hence leaving room for new scientific studies on the subject. To study natural products successfully, a suitable extraction process is paramount. The most popular techniques for isolating these aromatic essences are steam distillation and water distillation (Mwazighe, 2021). This study employed hydrodistillation using the Clavenger apparatus (Baj et al., 2015).

Gas Chromatography-Mass Spectrometry

Gas Chromatography-Mass Spectrometry (GC-MS) is a powerful hyphenated analytical technique that combines both gas chromatogram and mass spectrometer (Hussain & Maqbool, 2014). Chromatography is generally a separation technique that is usually applied in separating components in a mixture (Kugara et al., 2018). Chromatography, as a physical method of separation, in which the components to be separated are distributed between two phases, one of which is stationary phase while the other is mobile phase, move in a definite direction (Hussain & Maqbool, 2014). There are however several chromatographic techniques classified on the basis of the physical state of mobile phase (gas or liquid), chromatographic bed (column or planar), and mechanism of separation (ion exchange and size exclusion) (Kannan & Ph, n.d.). However, the working principle behind the said chromatographic methods is similar in that a solute is placed in a system that has both the mobile and the stationary phase, the solute then partitions between the two phases (BihShow Lou, 2014). The degree of interactions between the solute and the stationary phase determines the time taken (retention time) to elute or the distance moved from the base line (retention factor) (*Gas Chromatography Mass Spectrometry (GCMS)*, n.d.). These two stated factors; retention time and retention factor are used in identification of unknown compounds in a mixture by matching with retention time of standard compounds in computer libraries (Krishnaveni & Santhoshkumar, 2017). In this hyphenated instrument of analysis, gas chromatogram unit separates the components while the mass spectrometer detects the components and gives read-out information as a mass spectrum (Hussain & Maqbool, 2014).

On the other hand, mass spectrometry is used to characterize the components by identifying the molecular formula. When hyphenated with gas chromatography, the separated components enter the ionization chamber of the mass-spectrometer where they are ionized by either electron impact (EI) matrix-assisted laser desorption ionization (MALDI), chemical ionization (CI), plasma and glow discharge, electrospray ionization (ESI) laser ionization (LIMS), fast atom bombardment (FAB), and plasma desorption ionization (PDI)(Wang et al.,

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1996). In this research, electron impact ionization was used, where the vaporized molecules are bombarded with a stream of electrons. The high energy electrons at 70 eV have the potential to abstract an electron from the molecule forming a charged molecule known as the molecular ion. The molecular ion is usually at high energy and can break down into smaller charged molecules known as fragments. The charged ions are accelerated to the detector where they are analysed qualitatively based on their mass to charge ratio and then the computer record each analyte scans as a mass spectrum. GC-MS analytical technique has proved to be versatile and therefore has gained wide applications in quality control, research, analysis of essential oils, and industrial applications (Hussain & Maqbool, 2014). This is because the method is simple and gives accurate responses (Aziz *et al*, 2018). GC-MS was therefore used for both qualitative and quantitative analysis of essential oils.

MATERIALS AND METHODS

Sampling and Extraction of Essential Oils

5.000 kg of fresh leaves were collected during the rainy seasons when the plants were almost flowering as identified by Mr. Patrick Mutiso a taxonomist from the University of Nairobi. Flowers were deposited in the university of Nairobi herbarium under voucher specimen number as follows (JNM001/2021). Plant leaves of *S. manii* plant were collected from Kandara in Murang'a County Kenya. The fresh leaves were weighed using a spring balance. The fresh leaves were transported to the pharmacognosy laboratory at the Mount Kenya University. They were then washed with running tap water and rinsed with distilled water to remove any physical or chemical impurity. The leaves were later air dried on the benches at the Mount Kenya Pharmacognosy Laboratories for seven days to a constant mass of 0.5000 kg by losing 90% of the total moisture content. The total dry mass was weighed using an analytical grade balance Shimadzu ATY224 uniblock at the Pharmacy laboratory Mount Kenya University and the total dry mass of 0.5000 kg recorded. The percentage moisture content lost was computed by the following formula

$$Percenatge\ moisture\ lost = \frac{mass\ of\ fresh\ leaves - mass\ of\ dry\ leaves}{mass\ of\ fresh\ leaves}$$

The dry plant leaves from *S. manii*, were pulverized using a kitchen blender. 200.000 g of pulverized leaves were placed in 1000.000 ml round bottomed flask and 500.000 ml of distilled water added. The dry powdered plant materials were then hydrodistilled using the Clevenger apparatus for eight hours at 100℃ and ambient atmospheric pressure. The vial was weighed using Shimadzu ATY224 uniblock. The mass of essential oils was calculated by subtracting the mass of the vials from the mass of oils and the vials. The rate at which the oils were produced were computed as $0.00007Kg\ of\ essential\ oils/0.2Kg\ of\ dry\ leaves$ which is equivalent to 0.01%.

Analysis of Essential Oil extracts using Gas chromatograph-mass spectrometry analyses (GC-MS)

Essential oils *from S. manii* were diluted with a 1:4 mixture of pure n-hexane (non-polar solvent) and Dichloromethane (DCM-polar solvent) to 2% v⁄v for GC-MS analysis sample solution (Wei et al., 2018) and a sample solution of 1 µL was injected into GC-MS equipment using an auto-injector. Calibration of the equipment was done prior to analysis and time and temperature were determined and programmed at the time of analysis. GC-MS was used for the determination of both the qualitative and quantitative composition of the essential oils. Qualitatively the components were identified based on the retention index and by comparison with NIST-11 database (Wei et al., 2018). Quantitatively, the relative amounts of the constituents of the EOs was expressed as percentage by peak area approximated peak height approximation (Wang et al., 1996).

20 µL of the crude essential oils of *Solanecio manii* sample was diluted using 1 mL of a mixture of n-hexane and dichloromethane in the ration of 1:4. The component identification was achieved by Agilent 5977A MSD and 7890B GC system, Chemetrix; Agilent Technologies, DE (Germany) at the Government Chemist Department Laboratories in Nairobi County, Kenya. Helium was used as

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carrier gas at a constant flow of 1 mL/min and split less mode. An injector volume of 1 µl was employed with the mass spectra scanned from 40 to 560 m/z, at an injector temperature of 250 °C. Ionization was by electron impact (70 eV, source temperature 250 °C). The oven temperature was programmed from 50 °C (isothermal for 1 min.), with an increase of 10 °C/min, to 300 °C and held for 3 min Isothermal at 300 °C. Total GC running time was 32 min. Chromatographic separation was done using DP5-MS-UI.

RESULTS

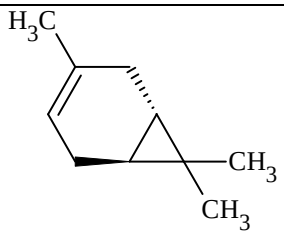
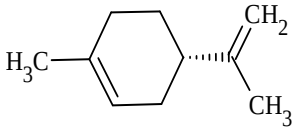
GC-MS Analysis

The components of essential oils were identified on the basis of comparison of their retention time and mass spectra with published data and computer matching with NIST11 and National Institute of Standards and Technology (NIST11) libraries (NISTEPA, NISTDRUG, and NISTTOX) provided with computer controlling the GC-MS system at the Government Chemist Department Laboratories, Nairobi County, Kenya. The spectrum of the unknown component was compared with the spectrum of the known components stored in the library. Peak areas were calculated and approximated using peak heights. The peak height is measured in mm and the heights summed up then percentages computed. According to (Wang et al., 1996) there is a 0.9993 correlation between the peak height and the concentration of the components of the mixture. The GC-MS analysis results for each of the essential oils are described below.

GC-MS Analysis of *S. manii* Essential Oils

The essential oils from *S. manii* revealed several peaks representing different compounds as shown in the total chromatogram by Gas Chromatography-Mass Spectrometry analysis. The peaks in the chromatogram were compared with the database of spectrum of known components stored in the Gas Chromatography-Mass Spectrometry library based on their retention index and the peak area determined peak height measurement methods. The retention times of the identified compounds matched with retention times of the compounds in the computer database libraries NIST11. Gas Chromatography-Mass Spectrometry analysis of the essential oils revealed the presence of different, terpene alcohol, bicyclic monoterpenes, cyclic monoterpenes, hydrocarbons, alcohols, and fatty alcohol see the *Appendix 1*. The results of GC-MS are described below in *Table 1* below. The natural products of *S. manii* identified by GC-MS were drawn using chemsketch software.

Table 1: Chemical constituents of the essential oil derived from S. manii by GC-MS analysis

Compound name	Retention time	%age	Molecular formula	Molecular structure	Classification
3-carene	8.2038	40%	$C_{10}H_{16}$		Bycyclic monoterpene
Limonene	7.9614	35.48%	$C_{10}H_{16}$		Cyclic monoterpene
1-undecanol	5.5285	8%	$C_{11}H_{24}O$		Fatty alcohol

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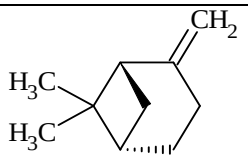
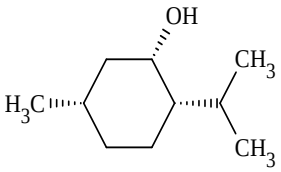
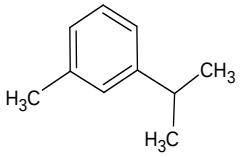
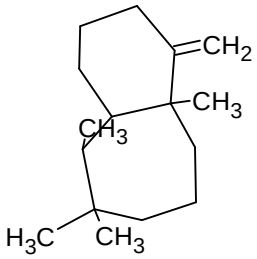
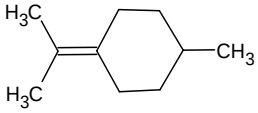
Compound name	Retention time	%age	Molecular formula	Molecular structure	Classification
β-pinene	7.2339	6.4%	$C_{10}H_{16}$		Bicyclic monoterpene
Menthol	18.5935	1.6%	$C_{10}H_{20}O$		Terpene alcohol
β-Cymene	7.8786	1.1%	$C_{10}H_{14}$		Aromatic organic compound
Longifolene	14.8445	0.78%	$C_{15}H_{24}$		Sesquiterpenoid
α-terpenolene	8.8951	0.5%	$C_{10}H_{16}$		Cyclic monoterpene

DISCUSSION AND CONCLUSION

Given that pedological factors influence the kinds of natural products that would be present in plants, samples from the same pedological zone (soils) were used. The plant's flowering season is the best time for natural product accumulation, samples were taken during this time. The GC-MS results confirm the findings of Bekele (1994) that terpenes are the most prominent constituents of plant essential oils. Terpenes derive their name from the word turpentine and they are most commonly found in the ethereal extracts of plants known as essential oils (BihShow Lou, 2014). There are over about 30,000 known terpenes all of them bearing isoprene unit (2-methylbut-1, 3-diene) as the most basic unit. Their classification therefore depends on the number of isoprene units. The following is the scheme of classification; hemi- (C5), mono- (C10), sesqui- (C15), di- (C20), sester- (C25), tri- (C30), tetraterpenes (C40) and polyterpenes (C5) n with n > 8. The analysis of the essential oils revealed the presence of different compounds that included, terpene alcohol, bicyclic monoterpenes, cyclic monoterpenes, hydrocarbons, alcohols and fatty alcohol (*1 Terpenes: Importance, General Structure, and Biosynthesis 1. 2 General Structure: The Isoprene Rule*, 2006). The compounds that were found present in the total ion chromatogram were, 3-Carene (40%), Limonene (35.48%), 1-undecanol (8.0%), β-pinene (6.4%), Menthol (1.6%), β-Cymene (1.1%) Longifolene (0.78%) and α-terpenolene (0.5%). This was however different from the findings of (Bernard Kiplangat Rono Tuei, 2019) which documents that the essential oils from

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S. manii were made of flavonoids, alkaloids and tannins. The difference may be due to different extraction method, different harvesting season and growth stage and the pedological difference from which the plant samples were obtained.

Recommendation

The study dwelled on hydrodistillation and gas chromatography-mass spectrometry analysis of essential oils. Future studies should focus other methods of extraction and analysis of the natural products present in *S. manii* leaves.

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Declaration of Competing Interest

The author declares that there are no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix 1