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CHARACTERISATION OF ESSENTIAL OIL FROM LEAVES OF Euphorbia heterophylla AND ITS INSECT REPELLENT POTENTIAL

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ABSTRACT

This study was designed to characterize the essential oil from leaves of *E. heterophylla* and its insect repellent potential. Essential oil from fresh leaves of *E. heterophylla* was extracted by hydro distillation method, using a Clevenger apparatus for 4 hours and the yield was 0.1816%w/w. Repellence test was conducted by using 5 cockroaches in number which were placed in a modified Y-tube Olfactometer and observations recorded for each and every odour introduced after 5 minutes in a relatively dark room. Repellency activity was computed in percentage form and it showed 100%. Analysis of the chemical constituents of the essential oil was done by Gas Chromatography coupled with a Mass Selective Detector, Ethyl benzene(4.58%), p-Xylene (2.22%),o-Xylene(2.73%), Nonane (1.70%), Benzene,1-ethyl-3-methyl-(1.09%), Benzene ,1,3,5trimethyl(1.09%), Decane (0.82%), Limonene (3.39%), Undecane (0.44%), 3-cyclohexen-1ol, 4methyl-1-(1-methylethyl)-(0.37%) and Dodecane (6.07%) as the constituents of the essential oil from *E.heterophylla* leaves. All these chemical constituents are active ingredients for insect repellants, thus this essential oil can be used as a natural based insect repellant to replace the synthetic insect repellants.

KEYWORDS: **Essential oil, Insect repellent potential, Insect repellant, DEET, GC-MS and *Euphorbia heterophylla***

INTRODUCTION

The control of vectors borne- diseases is becoming difficult because of resistance of vectors (insects) to insecticides over centuries hence imposing a threat to public health. As agents of chemical control, the use of insect repellents can be effective for protecting humans from cockroaches, mosquitoes, tsetse flies among others.[4]

Use of synthetic chemicals for example N, N-diethyl-m-methylbenzamide (DEET) in the control of cockroaches and other arthropods raises several concerns related to environment and human health. An alternative is to use natural products that possess good efficacy and are environmentally friendly. Among those chemicals, essential oils from plants belonging to several species have been extensively tested to assess their repellent properties as a valuable natural resource. [12]

Insect repellents contain agents that are used to protect the body from the bites of insects that can cause local or systemic effects. Whilst some bites cause only local skin irritation, some can cause serious illnesses and possibly death as the insects act as carriers or vectors of diseases. The insect repellents used currently fall into two categories; chemical repellents and natural plant-derived repellents. The most well-known and well-used chemical repellent is DEET (N, N-diethyl-3-methylbenzamide, previously called N, N-diethyl-m-toluamide). Other chemical insect repellents include IR3535, MGK-326 and MGK-264. The latest chemical agent proving to be as effective as DEET is a piperidine-based repellent called picaridin. (Vanessa & Staff, 2008

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https://www.dermnetnz.org/topics/insect-repellent/)

In addition, side effects of DEET like dizziness, skin irritations, nausea, headaches, mucous membrane irritation, difficulty concentrating and allergic reactions have been found when used in high concentrations, in areas of high temperature, applied to an exposed skin and wound cuts and age groups for example infants below two months, children under the age of 10 years although these reactions do not seem to present serious problems for DEET use. The adaptation of plant-based insect repellents is becoming increasingly popular because of their low toxicity but to date have not shown to be as effective as DEET. These include citronella, soybean oil and eucalyptus products. The metabolites like the monoterpenes such as α-pinene, cineole, eugenol, limonene, terpinolene, citronellol, citronellal, eugenol, camphor and thymol are the common constituents in a number of essential oils presenting vector-borne insect repellent activity . Generally, the monoterpenoids and sesquiterpenes are associated with repellent properties of several essential oils. [3]

Essential oils from *Euphorbia Heterophylla will* be characterized and used for insect repellency potential applications. The yield of *Euphorbia Heterophylla* essential oil will be done by using the hydro distillation method because it is simple to construct, faster, efficient, yields high amounts of the oil and it is suitable for small scale production of the oil.(The other scope of this research work is to investigate the active ingredients in the essential oil to prove its potential to be used as an insect repellent and to observe the repellent activity of insect towards the essential oil of *E. Heterophylla* using a modification of a Y-tube Olfactometer. This is because Y-tube Olfactometer gives choice to test between two or more sources of odor and also it was the first method which was used to provide the first experimental proof of insect response to plant odors. [5]; [11].

MATERIALS AND METHODS

Sample collection and identification

The plant leaves were collected from a garden around Mbarara University of Science and Technology, Uganda at a location of ±3m, S00.61440^0, E030.65456^0 obtained by a GPSmap76CSx model.

Identification was done by Dr. Eunice Olet, botanist at Mbarara University of Science and Technology, Department of Biology, where a sample voucher number, 001 was assigned and the specimen deposited in the herbarium.

Sample pretreatment methods

The collected fresh leaves *of Euphorbia heterophylla* were washed with tap water to remove the dirt on the surface of the leaves, this was to make sure that no any other impurities stick to the *Euphorbia heterophylla* leaves. The excess water moisture on the leaves' surfaces was then absorbed using paper towel. The extraction was carried out using only fresh leaves because maximum yield of essential oils are obtained than when dried leaves are used.

Isolation of the essential oils from leaves of E. *heterophylla*

The essential oils from fresh leaves of *E. heterophylla* were extracted using a modification of the established procedure [9].

250 g of fresh leaves of *Euphorbia heterophylla* were mixed with 1000 mL of distilled water in a 2 litres round bottom flask, after 4 hours of distillation, the essential oil were then removed from the water surface sing the Clevenger apparatus.

Pure essential oil samples were sealed and kept in a sample bottle at 9^o C in a refrigerator.

The EO obtained were then weighed and the yield was calculated as percentage of fresh starting plant materials using the formula below;

$$\text{Percentage of essential oil} = \frac{essential\ oil\ weight}{sample\ \ weight} \times 100$$

Repellency test

The repellence of the EO was evaluated using the method by [2] in the extraction of essential oil from Murraya koenigii leaves; potential study for application as natural-based insect repellent were the instrument that has been modified from Y-tube Olfactometer was used. This modification of the Y-tube Olfactometer was made using the dimensions of 10.2 cm for the stem,

This instrument was made from a transparent plastic glass made of Perspex fabricated with a shape of Y. The dimensions were as follows; stem-10.2cm, arms-15.1cm, internal diameter-6.5cm, width of arms-5cm and angle of 55°.
The test was done by using cockroaches (Periplaneta Americana (L.) (5 in number), in accordance to Jamil *et al.,* 2016.The observation during the test was recorded in the table. The percentage of repellency was then calculated basing on the formula:

% Repellence = $\frac{C-T}{C} \times 100$ where C is the

total number of cockroaches that lands on the control and T is the number of cockroaches that land on the treated area of the essential oil of *E.heterophylla*.

Table 1: Observation of the repellency test

Parameter	Observation
Cockroach only	
Cockroach with some biscuit	
Cockroach with repellent (essential oil)	

Gas Chromatography-Mass spectrometry (GC-MS) analysis of essential oils

GC-MS analysis of the essential oil was carried out on an Agilent model GC 6890N coupled with a Mass Selective Detector 5973B with a fused silica HP-5ms column (30m x 0.25mm x 25 μm) and helium as a carrier gas at constant pressure and flow rate of 3.3245cm3/min. The GC oven was initially programmed at 50 °C (hold for 1 min) and finally 300 °C (hold for 5 min) at a rate of 8°C/min when the trial temperature is 37.25°C. For MS, electron impact ionization was achieved with ionization energy of 70ev.The column heater was later set at 250°C while the pressure of 10.143 psi with an average velocity of 66.45cm/sec and hold-up time of 0.75245 min was recorded. The essential oil was diluted with diethyl ether and 2 μl of diluted sample was injected automatically in the splitless mode. This is available in Chemistry Laboratory, Chemistry Department at College of Natural Sciences (CONAS), Makerere University Uganda.
[8]; [2] and [10].

Identification of chemical components of essential oil of E. *heterophylla*

Identification of the chemical constituent compounds was done by the Chem. office software along with the MS-library. The individual chemical constituents were identified by their retention time identical to the compounds known from the literature data and also by comparing their spectra with those stored in the NIST0.8/Database.

RESULTS AND DISCUSSION

Yield of essential oil

250g of leaves of E.heterophylla were weighed and 0.454g of the essential oils were obtained The percentage yield of essential oil from leaves of essential oil from E.heterophylla was calculated as follows using the formula below;

Percentage of essential oil = $\frac{essential\ oil\ weight}{sample\ weight} \times 100$

$= \frac{0.454}{250} \times 100$ = 0.1816%w/w

Repellency test

The test was done at an interval of 5 minutes for every parameter in a relatively dark environment.

A complete repellent test was determined by the use of cockroaches and the observation of the repellency throughout the test was summarized in the table 2 and the evidence of the experiment on repellence in the figures below;

Table 2: Summary of the repellence test

Parameter	**Observation**
Cockroaches only	All the cockroaches stayed at different position (all sides of the Y-shaped transparent perspex.
Cockroaches with some biscuit	Some cockroaches went to the biscuit side (positive control) and others to the arm and stem of the Y-shaped transparent perspex.
Cockroaches with repellent (essential oil)	All cockroaches moved away from the essential oil to the arms of the Y-tube Olfactometer.

Figure 1: Repellency test

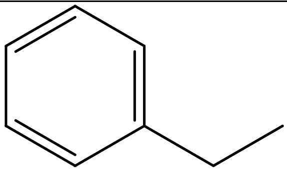
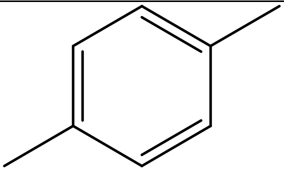
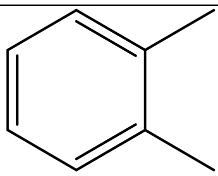
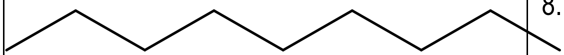
Repellency activity

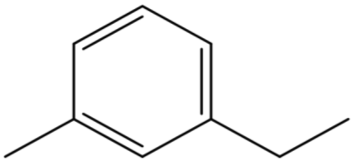
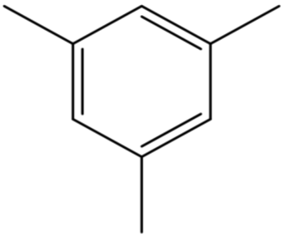
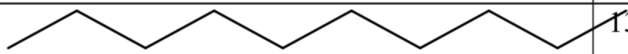
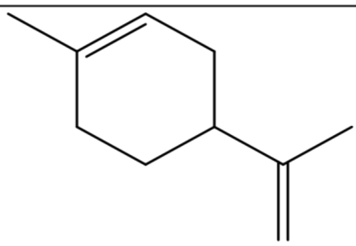
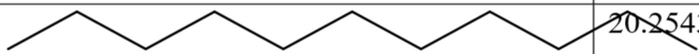
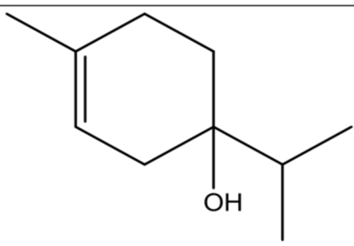
The repellence activity was carried out using 5 cockroaches in number to prove the feasibility of essential oil from E.*heterophylla* to repel insects. The test was performed using a modified Yshaped transparent perspex as shown in figure 9 above that was a modification of a Y-tube Olfactometer. The percentage repellency was calculated using the formula below;

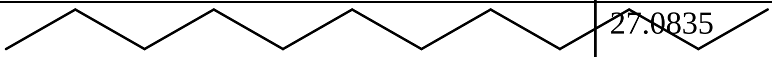
$$\text{Percentage repellency} = \frac{5-0}{5} \times 100$$
$$= 100\%$$

Analysis by Gas Chromatograph-Mass spectrometer

Table 3: Results from GC-MS analysis

S/N	CHEMICAL COMPONENTS	PERCENTAGE COMPOSITION	STRUCTURE	RETENTION TIME (MINUTES)
1	Ethyl benzene	4.58	 Ethyl benzene	6.4923
2	p-Xylene	2.22	 p-Xylene	6.8096
3	o-Xylene	2.73	 o-Xylene	6.8118
4	Nonane	1.70	 Nonane	8.1327

5	Benzene,1 -ethyl-3-methyl-	1.09	 Benzene,1-ethyl-3-methyl-	13.1385
6	Benzene ,1 ,3,5-trimethyl-	1.09	 Benzene ,1,3,5-trimethyl-	13.1385
7	Decane	0.82	 Decane	13.6790
8	Limonene	3.39	 Limonene	15.3363
9	Undecane	0.44	 Undecane	20.2543
10	3-cyclohexen-1-ol,4-methyl-1-(1-methylethyl)-	0.37	 3-cyclohexen-1-ol,4-methyl-1-(1-methylethyl)-	25.2781

	(terpinen-4-ol)			
11	Dodecane	6.07	 Dodecane	27.0835

DISCUSSION

The yield of essential oil from leaves of .heterophylla was 0.1816%w/w. The low yield was expected since leaves of plants are said to possess low essential oils compared to the flowers. However, this yield differs slightly from what [8] obtained from the leaves which was 0.21% w/w. This could be attributed to external factors such as climate, nature of the soil, age of the plant, and time of collection but also mode of extraction [1]. Furthermore, the factors that determine essential oil yield and composition are numerous, in some cases, also the seasonal variations, genetics and plant organ matter and therefore it is difficult to isolate these factors from each other as they are interrelated and influence each other[7].

The laboratory repellency results indicated that the volatile chemical components in the essential oils can repel cockroaches at a percentage of 100. This is because of the three experiments which were conducted using different odors in the Y-tube olfactometer.
The GC-MS analysis showed Ethyl benzene (4.58%), p-Xylene (2.22%),o-Xylene(2.73%), Nonane (1.70%), Benzene,1-ethyl-3-methyl-(1.09%), Benzene ,1,3,5-trimethyl(1.09%),Decane (0.82%), Limonene (3.39%), Undecane (0.44%), 3-cyclohexen-1-ol, 4-methyl-1-(1methylethyl)(0.37%) and Dodecane (6.07%) as the constituents of the essential oil from E.heterophylla leaves. Interestingly all the chemical constituents characterized were active ingredients responsible for insect repellency. However the concentration of the chemical components of E.heterophylla oil is very low but with a great insect repellency effect.

However, the results from the GC-MS analysis indicate chemical constituents which are dissimilar to that of Adedoyin *et al.,* 2013. Even though the methods may be the same, but also the source of raw materials that grows in various environments will gave inconsistent properties that have contributed to the results obtained in the research [2].

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