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Pharmacognostical characterization and formulation of herbal-based low-cost mosquito repellents from *Elettaria cardamomum* (Linn.) seed by using natural binder

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Abstract

Background: In the present scenario, the markets are flooded with chemical-based mosquito repellent products that are proven to be toxic to the human life especially to the children and quite expensive in term of cost. This present investigation was attempted to evaluate the complete pharmacognostical characteristics of *Elettaria cardamomum* (Linn.) seed and to formulate eco-friendly and cost-effective herbal mosquito repellents of the seed powder by using a natural binder.

Results: The results of microscopic study of seed confirmed the presence of perisperm, embryo, endosperm and testa (Figs. 4, 5 and 6). The extractive values, moisture contents and ash values were also analysed and found to be alcoholic extract, 4.88% w/w; water extract, 40% w/w; moisture content, 8.6%; total ash, 5% w/w; acid insoluble ash, 1.5% w/w; and water soluble ash, 3.5% w/w (summarized in Tables 1, 2 and 3). From the mosquito repellency test (Table 6), the combination of cardamom seed cake was done with cardamom powder 3/2 + 1/4 spoons, wood powder 3 spoons and chilli powder 1/4 spoon which showed low residual percentage of 15.05%, no irritation and an average burning time of 65 min. The formulated herbal-based mosquito repellents were found to be more effective without any side effects and found less cost too.

Conclusion: All the ingredients used in the formulation were herbal based and has no side effect on human health. The cost of the cake was Rs. 11.332/- and it can be considered as good mosquito repellent cakes because of its consistent burning ability with no irritating smoke and low residual percentage and high mosquito repellent ability. By utilizing this concept, the researchers can develop herbal, eco-friendly and cost-effective mosquito repellents in future.

Keywords: *Elettaria cardamomum* (Linn.) seed, Pharmacognostical evaluation, Mosquito repellent, Herbal based, Cost-effective

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Background

Mosquitoes are belonging to the family Culicidae of the order Diptera, and they are similar in physical characteristics to other flies. However, many important characteristics of anophelines (*Anopheles* spp.) differ from the culicinae [1, 2]. Repellents make a mosquito unappealing to humans, so that parts of the body that have been handled with the chemical are avoided. Mosquitos are not destroyed by repellents. With a single application, the better repellents can provide protection from bites for a prolonged period of time (> 1 h). Many plants produce compounds that are used by phytophagous (plant eating) insects to avoid attacks. These chemicals, including repellents, feeding deterrents, contaminants and growth regulators, fall into several groups. The majority can be classified into five main chemical categories: nitrogen compounds (primarily alkaloids), terpenoids, proteinase inhibitors, phenolics and growth regulators [3]. The currently available repellents are either synthetic chemicals such as DEET-N,N-Diethyl-meta-toluamide, picaridin and IR3535-Insect repellent IR3535, or chemicals extracted from plants such as citronella and eucalyptus lemon oil. There are different formulations available for these repellents that vary in the volume of the active ingredient, which is the material that actually repels the mosquito [4]. Insect repellents in different types and concentrations are available. For skin applications as well as for clothing care, aerosol and pump-spray items are intended. Products with liquid, cream, lotion, spray and stick allow direct skin application. For circumstances where exposure to insects is limited, items with low concentrations of active ingredients may be suitable. In heavily infested areas or with insect species that are more difficult to repel, higher concentrations of active ingredients may be useful [5]. *Elettaria cardamomum* (cardamom) is an evergreen shrub in the Zingiberaceae family (Fig. 1). As a spice, the seeds are used. It is regarded as the queen of spices. It ranks third in the worldwide list of expensive spices, followed by saffron and vanilla. Cardamom's medicinal usage is also listed in the Unani and Ayurveda systems. Cardamom also has a broad range of therapeutic characteristics such as antifungal, antibacterial, antiviral, diuretic and carminative properties. In addition, it is also used to treat heart failure, problems with the liver, anorexia, asthma and bronchitis. It is used as a spice and also finds use as medicine in culinary preparations and confectionery [6].

Methods

Collection and identification of plant material

The seeds were collected from the local market of East Sikkim. Then, it was identified and confirmed by following the standard literature studies. They were shade dried. The dried samples were ground to powder using electric blender.

Pharmacognostical examination

The macroscopical observations were carried out as per the standard methods to determine the shape, size, taste, odour and colour [7–10].

Microscopical examination

Transverse section of seed

Seed of the plant was sectioned by using blade; then, a clean glass slide was taken and a drop of glycerine water was placed in the centre of slide. In this, the section of seed was placed. The cover slip was placed by using the finger and thumb of the left hand and the edge of the cover slip was rested on the slide at the left hand edge of the drop. A dissecting needle was inserted under the right hand edge of the cover slip and the latter was rest on the needle. The cover slip was placed slowly onto the

Fig. 1 *Elettaria cardamomum* (Linn.) seeds

drop so that the liquid exactly fills the space between the slide and the cover slip without any air bubbles being trapped inside. The slide was placed in position on the stage of microscope. The T.S of the seed was seen by using × 10 and × 45 lens [11].

Extractive value

The extracts obtained by exhausting crude drugs are indicative of approximate measures of their chemical constituents. Taking into the consideration the diversity in chemical nature and properties of contents of drugs, various solvents are used for determination of extractive values [12].

Water soluble extractive

Method

About 5 g of the coarse powdered air-dried seed was macerated with 100 ml of chloroform water (2.5 ml in 1000 ml water) in a stoppered flask for 24 h. The content was shaking frequently for 6 h. It was filtered through filter paper taking precaution against excessive loss of solvent. Twenty-five millilitres of water extract was evaporated to dryness in a tarred flat bottomed shallow dish. It was dried at 105 °C and weighed. The percentage w/w of water soluble extractive value was determined with reference to the air dried drug [13].

Alcohol soluble extractive

Method

About 5 g of the coarsely powdered air-dried seed with 100 ml of alcohol (95% ethanol) in a stopper flask for 24 h. The content was shaking frequently for 6 h. It was filtered through filter paper taking precaution against excessive loss of alcohol. Twenty-five millilitres of water extract was evaporated to dryness in a tarred flat bottomed shallow dish. It was dried at 105 °C and weighed. The percentage w/w of alcohol soluble extractive value was determined with special standard reference to the air dried drug [14].

Determination of moisture content

Method

About 5 g of the air-dried crude drug was accurately weighed in a tarred watch glass. The drug was kept in hot air oven at 105 °C and dried for a period until constant weight obtained. The difference in weight gives the moisture content of the drug [15–17].

Determination of ash value

Total ash: method A

Two to 3 g of air-dried drug was weighed accurately in a tarred platinum or silica dish and incinerated at a temperature not exceeding 800 °C until free from carbon [18]. It was cooled and weighed. If a carbon free ash is not obtained, the charred mass was washed with hot water, the residue was collected on an ashless filter paper, and the residue was incinerated along with the filter paper until the ash is white or nearly white; the filtrate was added to the dish, evaporated to dryness. The percentage of total ash of the drug was calculated.

Acid-insoluble ash: method B

The ash (obtained from method A) was boiled with 25 ml of 2 M HCL acid for 5 min, and the insoluble matter was collected in a Gooch crucible or on an ashless filter paper; it was washed with hot water, ignited, cooled in a desiccators and weighed. The percentage of acid-insoluble ash was calculated on the dried drug basis [19].

Water soluble ash: method C

The ash (of method A) was boiled with 25 ml water for 5 min, and the insoluble matter was collected in a Gooch crucible or on an ashless filter paper, it was washed with

Fig. 2 Preparation of *Elettaria cardamomum* seed cake and drying

Table 1 Average wet and dry weights of cardamom seed cakes

Sl. no.	Name of the sample	Average wet weight (g)	Average dry weight (g)	Amount lost (g)
1.	Cardamom powder 2 spoons Wood powder 2 spoons Chilli powder 1 spoon	(i) 50.48	(i) 33.20	19.44
		(ii) 52.03	(ii) 30.35	
		(iii) 53.68	(iii) 31.22	
		(iv) 48.05	(iv) 30.85	
		(v) 50.70	(v) 32.10	
		Avg. 50.98	**Avg. 31.54**	
2.	Cardamom powder 1 spoon Wood powder 2 spoons Chilli powder 2 spoons	(i) 38.30	(i) 25.20	12.09
		(ii) 47.45	(ii) 36.82	
		(iii) 42.24	(iii) 27.92	
		(iv) 45.32	(iv) 30.13	
		(v) 40.57	(v) 33.34	
		Avg. 42.77	**Avg. 30.68**	
3.	Cardamom powder 2 spoons Wood powder 1 spoon Chilli powder 2 spoons	(i) 50.27	(i) 38.92	12.40
		(ii) 51.35	(ii) 38.57	
		(iii) 48.92	(iii) 36.32	
		(iv) 49.33	(iv) 37.43	
		(v) 51.24	(v) 37.90	
		Avg. 50.22	**Avg. 37.82**	
4.	Cardamom powder 3 spoons Wood powder 1 spoon Chilli powder 1 spoon	(i) 46.98	(i) 34.01	13.40
		(ii) 47.54	(ii) 32.20	
		(iii) 45.02	(iii) 32.24	
		(iv) 47.25	(iv) 33.28	
		(v) 46.30	(v) 34.33	
		Avg. 46.61	**Avg. 33.21**	
5.	Cardamom powder 1 spoon Wood powder 3 spoons Chilli powder 1 spoon	(i) 38.32	(i) 28.52	10.31
		(ii) 37.34	(ii) 27.21	
		(iii) 38.55	(iii) 25.72	
		(iv) 36.22	(iv) 28.41	
		(v) 37.30	(v) 26.33	
		Avg. 37.54	**Avg. 27.23**	
6.	Cardamom powder 1 spoon Wood powder 1 spoon Chilli powder 3 spoons	(i) 40.01	(i) 23.44	13.73
		(ii) 45.42	(ii) 32.95	
		(iii) 38.30	(iii) 33.51	
		(iv) 42.21	(iv) 28.62	
		(v) 46.52	(v) 25.31	
		Avg. 42.49	**Avg. 28.76**	
7.	Cardamom powder 3 spoons Wood powder 3/2 spoons Chilli powder 1/2 spoon	(i) 49.05	(i) 34.42	14.38
		(ii) 45.57	(ii) 33.40	
		(iii) 48.32	(iii) 32.23	
		(iv) 49.27	(iv) 34.54	
		(v) 47.53	(v) 33.24	
		Avg. 47.94	**Avg. 33.56**	
8.	Cardamom powder 7/2 spoons Wood powder 1 spoon Chilli powder 1/2 spoon	(i) 53.02	(i) 38.44	14.90
		(ii) 52.25	(ii) 37.54	

Table 1 Average wet and dry weights of cardamom seed cakes *(Continued)*

Sl. no.	Name of the sample	Average wet weight (g)	Average dry weight (g)	Amount lost (g)
		(iii) 52.22	(iii) 38.45	
		(iv) 53.34	(iv) 36.33	
		(v) 50.33	(v) 35.90	
		Avg. 52.23	**Avg. 37.33**	
9.	Cardamom powder 3/2 spoons Wood powder 3 spoons Chilli powder 1/2 spoon	(i) 42.23	(i) 28.14	14.66
		(ii) 40.43	(ii) 25.34	
		(iii) 43.25	(iii) 28.52	
		(iv) 42.52	(iv) 27.99	
		(v) 41.61	(v) 26.73	
		Avg. 42.00	**Avg. 27.34**	
10.	Cardamom powder 1 spoon Wood powder 7/2 spoons Chilli powder 1/2 spoon	(i) 37.34	(i) 26.25	10.86
		(ii) 36.02	(ii) 27.12	
		(iii) 37.55	(iii) 26.26	
		(iv) 35.32	(iv) 24.33	
		(v) 37.41	(v) 25.37	
		Avg. 36.72	**Avg. 25.86**	
11.	Cardamom powder 2 spoons Wood powder 5/2 spoons Chilli powder 1/2 spoon	(i) 44.32	(i) 30.52	13.32
		(ii) 40.57	(ii) 28.10	
		(iii) 43.43	(iii) 27.25	
		(iv) 44.32	(iv) 32.34	
		(v) 42.73	(v) 30.55	
		Avg. 43.07	**Avg. 29.75**	
12.	Cardamom powder 5/2 spoons Wood powder 2 spoons Chilli powder 1/2 spoon	(i) 49.52	(i) 33.43	16.57
		(ii) 48.79	(ii) 30.55	
		(iii) 49.83	(iii) 33.72	
		(iv) 47.54	(iv) 32.35	
		(v) 48.32	(v) 31.12	
		Avg. 48.80	**Avg. 32.23**	
13.	Cardamom powder 5/2 + 1/4 spoons Wood powder 2 spoons Chilli powder 1/4 spoon	(i) 54.14	(i) 38.72	15.91
		(ii) 52.31	(ii) 37.57	
		(iii) 54.29	(iii) 38.49	
		(iv) 55.84	(iv) 37.62	
		(v) 53.50	(v) 38.12	
		Avg. 54.01	**Avg. 38.10**	
14.	Cardamom powder 2 spoons Wood powder 5/2 + 1/4 spoons Chilli powder 1/4 spoon	(i) 49.54	(i) 34.79	15.03
		(ii) 47.57	(ii) 33.59	
		(iii) 48.32	(iii) 34.70	
		(iv) 49.19	(iv) 32.24	
		(v) 49.24	(v) 33.42	
		Avg. 48.77	**Avg. 33.74**	
15.	Cardamom powder 3 spoons Wood powder 3/2 + 1/4 spoons Chilli powder 1/4 spoon	(i) 55.54	(i) 39.49	15.46
		(ii) 54.09	(ii) 38.51	
		(iii) 55.50	(iii) 39.03	
		(iv) 53.85	(iv) 40.82	

Table 1 Average wet and dry weights of cardamom seed cakes *(Continued)*

Sl. no.	Name of the sample	Average wet weight (g)	Average dry weight (g)	Amount lost (g)
		(v) 54.72	(v) 38.55	
		Avg. 54.74	**Avg. 39.28**	
16.	Cardamom powder 3/2 + 1/4 spoons Wood powder 3 spoons Chilli powder 1/4 spoon	(i) 42.54	(i) 28.79	12.62
		(ii) 40.38	(ii) 29.55	
		(iii) 43.05	(iii) 30.32	
		(iv) 42.55	(iv) 29.97	
		(v) 41.74	(v) 28.52	
		Avg. 42.05	**Avg. 29.43**	
				Avg. 14.06

hot water, and ignited for 15 min at a temperature not exceeding 800 °C. The weight of the insoluble matter was subtracted from the weight of the ash; water-soluble ash reflects the difference in weight. On the dried drug basis, the percentage of water soluble ash was then measured [20].

Preparation of cakes

Fifteen to sixteen cakes of different weight with different proportion of the components were prepared (Fig. 2). Wet weight of cakes was taken. For the determination of dry weight, cakes were allowed to dry in the sun for 96 h and dry weight was taken [21]. Natural binder (wood powder-mixed powder from Alnus, Abies tress) was purchased from local vendor. To increase the efficacy of the replicates cakes, chilli powder with various proportions was mixed and natural gum collected from gum Arabic tree from local area was used as adhesive.

Preparation of cardamom seed cakes in combination with natural binder

For the preparation of mosquito repellent coils, different fillers can be used; however, in this study, natural binder was utilized. Mosquito repellent cakes were prepared by plating total 5 table spoons (1 table spoon = 20 ml) of cardamom seed powder, chilli powder and wood powder with various proportion along with gum in thermocol plates [22, 23]. Wet weight of cakes and dry weight (after 96 h) of prepared cakes were recorded (Table 1). A significant weight loss is recorded, i.e. 14.06 g in all combinations.

Evaluation of cardamom seed cakes

The efficacy of prepared cardamom seed cakes were evaluated on three different parameters such as flammability, burning time and mosquito repellence test [24].

a) Flammability test and burning time

To observe the flammability of the cakes, the cakes were burnt using candles (Fig. 3). The quantity of ash, irritation produced by different combination of cakes and the time taken to burn completely were recorded (Table 2). Each combination's ash weights and their burning time have been documented [25]. Residual percentages were determined based on ash weight and dry weight. The percentage residual is determined by:

Fig. 3 Burning of *Elettaria Cardamomum* seed cake and its ash

Table 2 Parameters to check flammability of cardamom seed cakes

Sl No.	Name of the sample	Dry weight (g)	Ash weight (g)	Time taken to burn (min)	Residual (%)	Irritation	Remarks
1.	Cardamom powder 2 spoons Wood powder 2 spoons Chilli powder 1 spoon	31.54	5.58	80	17.69	High irritation	Fully burnt
2.	Cardamom powder 1 spoon Wood powder 2 spoons Chilli powder 2 spoons	30.68	5.76	75	18.77	Very high irritation	Fully burnt
3.	Cardamom powder 2 spoons Wood powder 1 spoon Chilli powder 2 spoons	37.82	6.62	78	17.50	Very high irritation	Fully burnt
4.	Cardamom powder 3 spoons Wood powder 1 spoon Chilli powder 1 spoon	33.21	8.45	83	25.44	High irritation	Not fully burnt
5.	Cardamom powder 1 spoon Wood powder 3 spoons Chilli powder 1 spoon	27.23	3.79	73	13.91	High irritation	Fully burnt
6.	Cardamom powder 1 spoon Wood powder 1 spoon Chilli powder 3 spoons	28.76	5.79	76	20.13	Very high irritation	Fully burnt
7.	Cardamom powder 3 spoons Wood powder 3/2 spoons Chilli powder 1/2 spoon	33.56	5.96	82	17.75	Less irritation	Fully burnt
8.	Cardamom powder 7/2 spoons Wood powder 1 spoon Chilli powder 1/2 spoon	37.33	8.02	97	21.48	Less irritation	Not fully burnt
9.	Cardamom powder 3/2 spoons Wood powder 3 spoons Chilli powder 1/2 spoon	27.34	4.25	80	15.54	Less irritation	Fully burnt
10.	Cardamom powder 1 spoon Wood powder 7/2 spoons Chilli powder 1/2 spoon	25.86	3.88	75	15.00	Less irritation	Fully burnt
11.	Cardamom powder 2 spoons Wood powder 5/2 spoons Chilli powder 1/2 spoon	29.75	5.62	84	18.89	Less irritation	Fully burnt
12.	Cardamom powder 5/2 spoons Wood powder 2 spoons Chilli powder 1/2 spoon	32.23	6.34	85	19.67	Less irritation	Fully burnt
13.	Cardamom powder 5/2 + 1/4 spoons Wood powder 2 spoons Chilli powder 1/4 spoon	38.10	8.77	95	23.01	No irritation	Not fully burnt
14.	Cardamom powder 2 spoons Wood powder 5/2 + 1/4 spoons Chilli powder 1/4 spoon	33.74	6.55	90	19.41	No irritation	Fully burnt
15.	Cardamom powder 3 spoons Wood powder 3/2 + 1/4 spoons Chilli powder 1/4 spoon	39.28	9.22	92	23.47	No irritation	Not fully burnt
16.	Cardamom powder 3/2 + 1/4 spoons Wood powder 3 spoons Chilli powder 1/4 spoon	29.43	4.43	65	15.05	No irritation	Fully burnt

$$\text{Residual}\ (\%) = \text{Ash weight}/\text{dry weight} \times 100$$

b) Mosquito repellency test

Mosquito repellency test was done by simply selecting the mosquito-prone areas in the evening and night period such as bushes, shrubs, laboratory corners and cafeteria [26] (Table 3). The public remarks were noted down after allowing them to burn the cakes and checking if the mosquitoes are present or escaping away from the burning cakes.

Table 3 Mosquito repellency test in different areas of Department of Zoology

Sl No.	Areas	Reports given by people	Remarks
1.	Department premises	Smoke caused irritation	Mosquito repelled
2.	Class room corners	Mosquitoes escaped	Mosquito repelled
3.	Cafeteria	Mosquitoes moved outside	Mosquito repelled
4.	Laboratory corners	Smoke caused irritation	Mosquito repelled

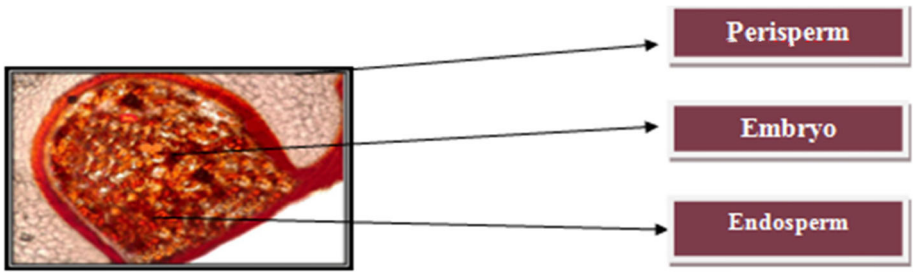

Fig. 4 TS of embryo

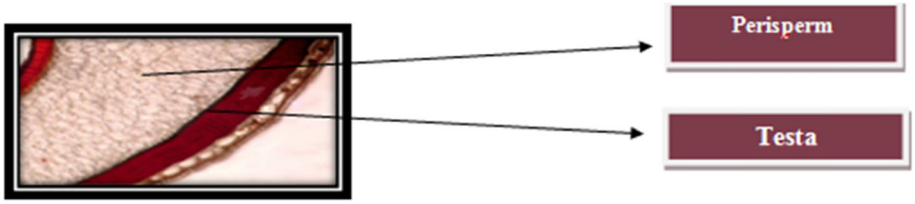

Fig. 5 T.S. of testa and perisperm

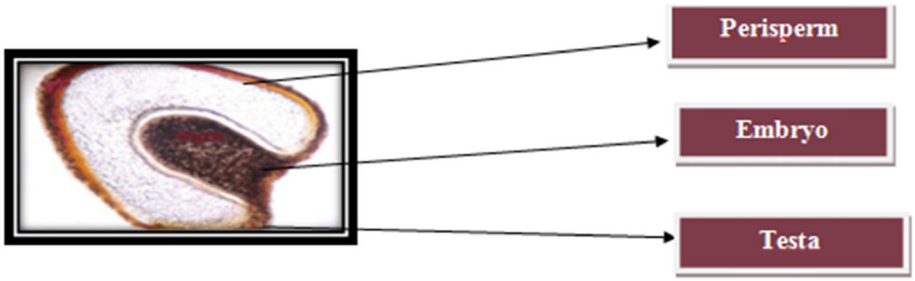

Fig. 6 Whole section showing testa, perisperm and embryo

Results

Pharmacognostical

The pharmacognostical results are shown in Figs. 4, 5 and 6.

Proximate analysis

The extractive values, moisture contents and ash values are shown in Tables 4, 5 and 6.

Discussion

Insects are also known for their aggravating characteristics of biting, stinging, contaminating and spreading disease, so people have long found ways to contain them. We do not need all insect life to be killed. Birds, reptiles and mammals are supplied with food by insects and several help pollinate plants [27]. Health stores sell a range of bug repellent based on essential oil that are sufficiently diluted to avoid skin irritation. Essential oils should be used carefully by pregnant women. The macroscopic analysis is a morphological summary of the naked eye or magnifying lens of the plant components. The microscopic research is the anatomical study conducted by taking a suitable part of the plant parts under analysis [28]. Each distinguishing characters are noted down. To obtain clear sections of TS phloroglucinol, HCL are used. Estimation of extractive values determines the amount of the active constituents in a given amount of plant material when extracted with a particular solvent. Extractions of any crude drug containing a specific solvent create a solution containing various phytoconstituents. The structures of these phytoconstituents depend on the essence of the drug and the solvent used. It also provides an indication of whether or not the crude drug is depleted. To prevent the growth of bacteria, yeast or fungi during storage, the moisture content of drugs should be at a minimum level. Ash values are used to determine crude drug content and purity. The presence of various impurities such as carbonate, oxalate and silicate is suggested. To estimate the amount of inorganic compounds found in medicines, water soluble ash is used. From the tests experiments, herbal-based mosquito repellents showed promising result of its consistent burning ability, less residual percentage and no irritation due to the use of natural wood powder, natural gum and combination of herbal ingredients.

Conclusion

Malaria, dengue fever and filariasis, which are transmitted via mosquito bites, are a major public health issue in tropical regions, especially in Africa and Asia. Therefore, for successful control of these diseases, individual mosquito bites need to be avoided. Instead of commercially available synthetic insecticides and repellents such as organophosphorus, carbamate, N,N-diethyl-3-methylbenzamide (DEET) and dichloro diphenyl trichloroethane (DDT), which are carcinogenic and non-eco-friendly, the use of mosquito repellent naturally obtained from medicinal plants is an efficient and healthy process. The use of synthetic chemicals to monitor insects and arthropods currently poses many environmental and human health issues. Using natural goods that have good effectiveness and are environmentally friendly is an option. Among those chemicals, essential oils from plants belonging to several species have been extensively tested as a valuable natural resource to evaluate their repellent properties. Further detailed research, however, needs to be carried out by collecting unique numbers of mosquitoes in a glass chamber covered with cloth sieve and exposing them to the smoke created by the herbal product with

Table 4 Extractive value

Sl. No	Type of extract	Percentage (W/W)
1	Alcohol	4.88%
2	Water	40%

Table 5 Moisture content

Weight of drug	Initial wt. of drug + petridish (g)	Constant wt. after drying (g)	Loss on drying (g)	Moisture content
10gm	64.92	64.06	0.86	8.6%

Table 6 Determination of ash value

Sl. no	%W/W	
1.	Total ash	5
2.	Acid insoluble ash	1.5
3.	Water soluble ash	3.5

varying concentrations and documenting mortality time and comparing them with chemical-based formula in the artificial. Because of its consistent burning ability with no irritating smoke and low residual percentage and high mosquito repellent power, it can therefore be considered good mosquito repellent.

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Authors' contributions
R.D, responsible for Selection of the research work and major data collection by trial and error methods. P.P, responsible for the guidance of the work till the end. S.B, contributed for drafting, designing, formatting and referencing of this research article and communicating with scientific esteemed journal having good reputation in the scientific fields. S.B plays centre part for the overall formatting and drafting of this scientific research article. All authors have read and approved the manuscript.

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