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Extraction & characterization of essential oils having mosquito repellent activity

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Abstract
Since they spread diseases including yellow fever, Dengue fever, filariasis, malaria, and others, mosquitoes rank high on the medical community's list of major disease vectors. The best approach to avoid these diseases at the moment is to reduce mosquito vectors and to take personal precautions against mosquito bites. People often use synthetic repellents like DEET, IR3535, and Picaridin to avoid mosquito bites. Many people are worried about the effects of synthetic repellents on the environment and human health. For this reason, we are paying close attention to essential oils (EOs) as a natural substitute. Naturally occurring repellents that are particularly effective against mosquitoes. The current research aims to extracts and characterized different essential oil having potential to repel the mosquito.

Keywords: DEET, essential oil, mosquito bites, dengue fever, environment

1. Introduction
Mosquitoes are among the most disturbing blood sucking insects afflicting human beings (Imeda C et al., 2013; Makhaik M et al., 2005). Several mosquito species belonging to genera Anopheles, Culex and Aedes are vectors for the pathogens of various diseases like Dengue fever, Malaria, Yellow fever, Japanese Encephalitis and several other infections (Adeogun AO et al., 2012). Mosquitoes alone transmit diseases to more than 700 million people and over one million deaths are reported annually across the globe (Mohomed AA et al., 2012; WHO, 2014). Therefore, the control of mosquitoes is an important public health concern around the world. As most of the mosquito repellent products and devices available in the market are reported to have harmful effects on human beings, the objective of the present study is to extract the plant-based essential oils which can serve as the effective mosquito repellent. Mosquitoes are small flies that belong to the family Culicidae. The mosquito is a Spanish word which mainly refers to “little fly” (Brown and Lesley, 1993). These are mainly invertebrates. About 3000 of mosquito’s species transmit disease when compared to other creatures in the world. Both female and male mosquitoes feed on the same kind of food. However, the female mosquitoes are only responsible for the transmission of the pathogens. The mosquito may cause life threatening diseases such as Filariasis, Dengue fever, yellow fever, Chikungunya and other arboviruses. They mainly transmit the diseases just by a bite on the skin and through its saliva that pathogens get injected into the host. Mosquitoes mainly transmit diseases to more than 700 000 000 people annually and which results for the death 1 out of 17 is alive (Carney et al., 2022; Poungjai S et al., 2011). Many of the synthetics and herbal medicines used in the treatment of diseases caused by the mosquitoes but the potential of essential oils against the mosquitoes and as the mosquitoes repellant agent is quite impressive. Many of the essential oils have effective repellency power against mosquitoes like *Coriandrum sativum* (Coriander oil), *Citrus aurantium* (Petitgrain oil), *Vetiveria zizanioides* (Vetiver oil), *Cymbopogon nardus* (Citronella oil) (Mishra P et al., 2023) , *Mentha piperita* (Peppermint oil), *Rosmarinus officinalis* (Rosemary oil), and many others (Sutthanont N, 2022) . The aim of the present study is to extract the essential oil from the plants which has the mosquito repellant activity.

2. Materials and Methods

Fresh thyme leaves (*Thymus vulgaris*) (Fig 1), rhizomes of nagarmotha plant (*Cypress rotundus*) (Fig 2, Fig 3), were collected within a different area in Agra and identified by a Botanist who assigned a Voucher Number (DB/H/1012) to the stored specimen at the Herbarium of the Raja Balwant Singh College Agra. The collected samples were stored in the laboratory at the optimum conditions and further used for the extraction of essential oil. The other samples like lavender oil and ylang-ylang oil purchased from the authentic supplier and evaluated for its authenticity.

Fig 1: Thymus vulgaris plant

Fig 2: Plant of Nagarmotha (*Cypress rotundus*)

Fig 2: Roots of *Cypress rotundus*

2.1 Extraction of Essential oil

The collected samples were chopped into the small pieces with the help of knife. The extraction for essential oil was done by using the Clevenger apparatus through the hydro- steam distillation method. The accurately weigh plant part used to submerged in the distilled water in the round bottom flask and connected to the condenser part. The extraction process carried out for each of the collected sample for the period of about 5-6 hours and yield of the extracted oil was calculated for each four samples. (Dehariya N et al., 2021)

% yield of essential oil= wt. of essential oil/initial wt. of plant material X 100

2.2 Characterization of extracted oils

2.2.1 Gas Liquid Chromatography

The GLC analysis was carried out QA Laboratory at FFDC Kannuaj. The Reference no. was FFDC/KNJ/QAL/2023-24 and the test method followed was IS:326 (Part 19) 1998RA2014.

2.2.2 Physiochemical analysis of Essential oil

The extracted essential oil evaluated for their various physical characters in order to evaluate their quality and identity. The refractive index of oil was determined by the refractometer at the temperature of 34℃. A drop of oil used to place in the Abbe refractometer and refractive index was determined. Specific gravity of the extracted oil was determined by the specific gravity bottles i.e., pyknometer. Specific gravity calculated by the formula –

Density= Weight of sample–weight of pyknometer/
weight of distilled water - weight of pyknometer

Optical rotation of the essential oils was determined by the instrument polarimeter. Essential oil used to put in the sample cell and align the light source by adjusting the knob of the analyzer (Shabbir MK, et al., 2009; Yadav SK, 2022).

3. Results and Discussion

The essential oil extracted from the plant samples and yield obtained was 1.77% for Thyme oil, and 1.31 % for nagarmotha oil.

3.1 Percentage yield of Extracted oil

Plant	Initial wt. of the plant (gm)	Wt. of essential oil obtained (gm)	% Yield
Thyme	700. 00	12.39	1.77
Nagarmotha	1,144.52	15.01	1.31

3.2 Chemical composition of essential oil

The essential oils extracted from the different plants samples and purchased samples analyzed by the gas liquid chromatography and different peaks in the GLC graph indicated the presence of volatile constituents. Linalool and Linalyl acetate (Fig 1) present in the Lavender oil at the concentration of 11.42% and 01.07% respectively. Thymol (Fig. 2) present in the concentration of 41.98% in the thyme oil and sharp peak indicate the presence of thymol in the GLC graph. Peak of Linalool (Fig 3) present in the Ylang-ylang oil at the retention time of 8.194 min. Cyprene (Fig. 4) as the main constituent present in the nagarmotha oil at the concentration of about 26.94%.

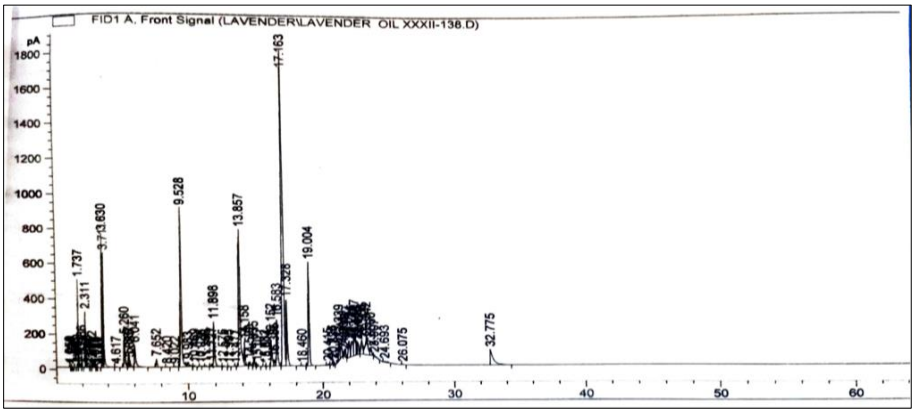

Fig 1: GLC graph of Lavender Oil

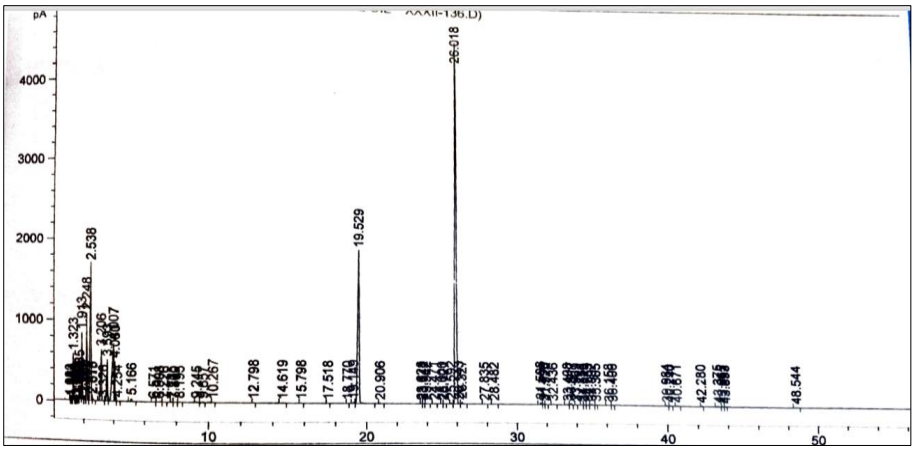

Fig 2: GLC graph of Thyme Oil

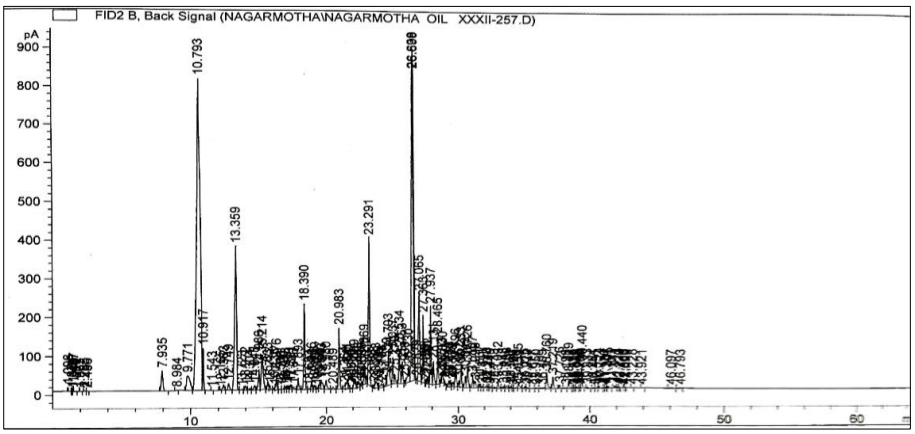

Fig 3: GLC graph of Nagarmotha Oil

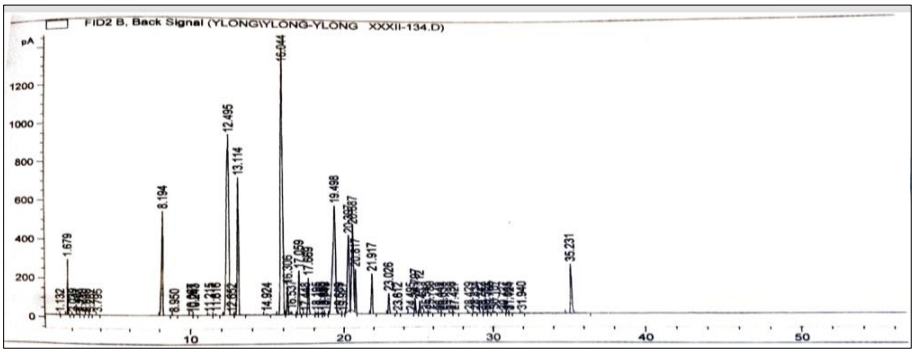

Fig 4: GLC graph of Ylang-Ylang Oil

3.3 Results for Physical parameters

The characterization of the essential oils was done by using the specified procedures as mentioned in Yadav SK, 2022. The lavender oil extracted from the leaves part was light yellow in color (Table 1) and showed the refractive index 1.464. The specific gravity of the lavender oil was 0.881 and the optical rotation was -11.1°. The thyme oil (Table 2) extracted from the leaves of *Thymus vulgaris* plant and the color of the extracted oil was reddish brown with the refractive index 1.497. The optical rotation of the oil was -4.7° and the specific gravity was found to be 0.920. The extracted ylang-ylang oil (Table 3) was colorless with the optical rotation of -17°. The refractive index of ylang-ylang oil was 1.498 and specific gravity was found to be 0.960. Nagarmotha oil extracted from the rhizome part was amber color (Table 4) with the optical rotation of -2.0°. The specific gravity of the oil was 0.960 and refractive index was 1.498.

Table 1: Results for physical parameters of Lavender oil

S. No.	Parameters	Results
1.	**Colour**	Light Yellow
2.	**Refractive index**	1.464
3.	**Specific gravity**	0.881
4.	**Optical rotation**	-11.1°

Table 2: Results for physical parameters of Thyme oil

S. No.	Parameters	Results
1.	**Colour**	Reddish brown
2.	**Refractive index**	1.497
3.	**Specific gravity**	0.920
4.	**Optical rotation**	-4.7°

Table 3: Results for physical parameters of Ylang-Ylang oil

S. No.	Parameters	Results
1.	**Colour**	Colourless
2.	**Refractive index**	1.498
3.	**Specific gravity**	0.960
4.	**Optical rotation**	-17°

Table 4: Results for physical parameters of Nagarmotha oil

S. No.	Parameters	Results
1.	**Colour**	Amber Colour
2.	**Refractive index**	1.510
3.	**Specific gravity**	0.960
4.	**Optical rotation**	-2.0°

Conclusion

The essential oils extracted from the different sources consist of the volatile constituents as like linalool, cyprene, linalyl acetate and thymol. The purchased samples also indicate the results within the specified range which confirms their authenticity. Many of the researches conducted to evaluate the repellency activity of essential oil against the mosquitoes as linalool has good repellency power against mosquitoes (Mishra P et al., 2023). The yield of the essential oils collected from the different sources was evaluated. The characterization of oil shows the results within the range as specified in the ISO guidelines.

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