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# Effective mosquito repellent from plant based formulation

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**Abstract**
All over the developing countries in the tropical region mosquito menace has been very acute leading to many diseases. Many chemicals have been used for the purpose of Mosquito repellency or killing, however they are extremely harmful for human beings as well. Recently, commercial repellent products containing plant-based ingredients have gained increasing popularity among consumers, as these are commonly perceived as "safe" in comparison to long-established synthetic repellents. Our paper describes Plant-based repellents for mosquitoes for personal protection measures. Based on the knowledge on traditional repellent plants obtained through ethnobotanical studies, development of new natural products is the need of the hour. The product developed that offers high repellency as well as good consumer safety. Thus this plant-based repellent made from 5 plant leaves has promising results in the field study.

**Keywords:** Mosquito repellent, lantana, Calotropis, lemon grass, Tulsi and neem, Aedes aegypti mosquito

## Introduction
How to control of mosquitoes in temperate climatic condition when so many mosquito bitten diseases are happening in present times is something of utter significance with rising number of mosquitoes in their breeding season. In the summer months, in the Asian countries, the mosquitoes can be more a nuisance not only by their bite, but even by their sound. Mosquitoes need to be exterminated using preferably by using non-hazardous chemicals. On the other hand, many of the pest control make chemical choices for getting rid of mosquitoes are extremely harmful for us. These chemicals that are not good for us as these mosquito control products are not a green choice.

Their control on breeding is of utmost importance. So that the mosquito control measures can be successfully carried out both in urban and rural areas. First and foremost thing is to destroy the breeding areas of these mosquitoes. One of the primary reasons for their worldwide spread is due to deforestation and industrialized farming. They are causing an alarming increase in the range mosquitoes [1]. The World Health Organization blames global warming for the expanding range of mosquitoes that are responsible for malaria, yellow fever and dengue fever, causing millions at risk. It is estimated by WHO nearly 15,000 deaths per year at all ages occur only in the Indian Peninsula [2]. Although chemical mosquito repellents available in the market have been formulated so that they have remarkable safety profile, but their toxicity against the skin and nervous system can cause rashes, swelling, eye irritation and other serious problems to children. Hence it was felt that bio-based natural mosquito repellents would be preferred over chemical mosquito repellents.

Bio based mosquito repellent are pest management tools that are based on safe, biologically based active ingredients derived from plants. Benefits of bio-products include effective control of mosquito as well as human and environmental safety. These bio based products were designed to play an important role in providing pest management tools in areas where mosquito resistance, niche markets, and environmental concerns limit the use of products. Mosquito repellents from natural sources are not new. Some of these mosquito repellent are derived from the following--Basil (Ocimum *basilicum*), oils of Castor, Cedar, Clove, Fennel, Citronella [3], Eucalyptus, Neem [4, 5, 6], Rosemary and Catnip oil of Nepeta species having nepetalactone, Celery extract (*Apium graveolens*) as well as *Solanum villosum* berry juice. These natural resources are good for the environment and also have added benefit of smelling good.

The attraction of mosquitoes towards human beings is due to the lactic acid and $CO_2$ which is present in our sweat [7]. The chemoreceptors present in the antennae of mosquitoes perceive the smell of the sweat. The role of natural mosquito repellent is to mask human scent. Controlling mosquitoes is of utmost importance in the present day scenario with rising number of mosquito borne diseases. An alarming increase in the range of mosquitoes is mainly due to deforestation, industrialized farming and stagnant water [8]. Thus, special products like mosquito repellents for combating mosquitoes are required. The products used for mosquito repellency show varying degrees of effectiveness. A number of natural mosquito repellents were studied in many research papers and review papers [9, 10, 11, 12] that work to repel mosquitoes. Mosquito repellents based on chemicals has a designed with remarkable safety profile, but even then they are toxic against the skin and nervous system and usually cause rashes, swelling and sometimes eye irritation. Hence, natural mosquito repellents are preferred over chemical products.

The potential of volatile oils extracted from turmeric, citronella grass and hairy basil as topical repellents against both day- and night-biting mosquitoes has been demonstrated [11]. In the case of Citronella species, for example, the components present in the oil are responsible for the desirable repellent characteristics provided only 2-3 hours of repellency. Most of the essential oil-based repellents tend to give short-lasting protection for less than three hours [12]. The smell characteristic of the oil comes from the presence of four main components, citronellal, eugenol, geraniol and limonene. Our attempt in this study was to use the leaf extracts of lantana, lemongrass, tulsi, calotropis and neem hoping to show synergistic effectivity in mosquito repellency. So we used equal proportion of each of the leaves and found that the duration of the effectivity was enhanced to 5-6 hours. This effectivity could be attributed to the vast range of terpenoids present in these plant extract. These volatiles have high vapor toxicity for mosquitoes. As is very well documented that—

The phytochemical analysis of leaves of Calotropis(Madar) show presence of Uscharin, Calotoxin, calctin, calotropeol acetate, β-amyrin acetate, giganteolacetate, mudarol along with citrates, chlorides and tartarates of sodium and potassium.

Lantana (*Lantana camara* L.) Lantana oil and crude extract are used as natural fumigants against many insects and mosquitoes. Leading phytochemical compounds in *Lantana* include mainly (triterpenoids), Oleanonic acid, Lantadene A, Lantadene B, Lantanilic acid, icterogenin and 4,5- dihydroxy-3,7-dimethoxyflavone-4-o-beta D- glucopyranoside, Camaroside. These compounds were responsible for its repellency activity.

Lemongrass (*Cymbopogon citrates. L*) the leaves have a lemon like odor because they contain an essential oil having citral as main constituent, same as what is present in lemon peel. Leading phytochemical compounds in lemongrass leaf are terpineol, Dipentene, Limonene, α-terpineol, citronellol, methyl heptenone, dipentene, geraniol, limonene, nerol, farnesol, mainly (triterpenoids).

Tulsi (*Ocimum sanctum* L.) is an aromatic herbs indigenous to the tropical regions of Asia. *Ocimum sanctum* is little known in the Western world but wildly cultivated in India. Leading phytochemical compounds in holy basil leaf include eugenol (volatile oil), ursolic acid (triterpenoid) and rosmarinic acid (phenylpropanoid). Other active compounds include caryophyllene and oleanolic acid. Seeds contain fixed oils having linoleic acid and linolenic acid.

**Fig 1:** Lantana camara

**Fig 2:** Lemongrass

**Materials and Methods:** Collection of leaves of Lemon grass (Cymbopogon *citrates* L.), Lantana, Calotropis and Neem were collected from Horticulture Department of IIT Kanpur, India. 175 gms of air dry lemon grass material were taken, after soxhlet extraction and evaporation of the solvent yielded 57 gms of the extract as shown in Fig-3.

**Fig 3:** Neem leaves

**Fig 4:** Calotropis leaves

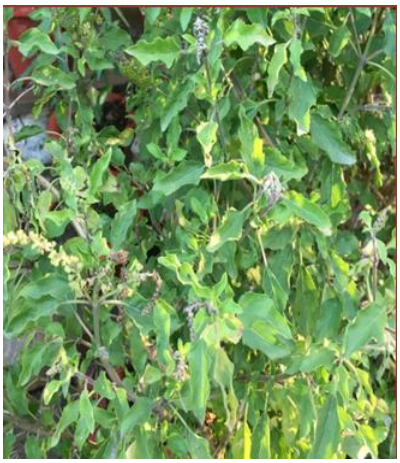

**Fig 5:** Tulsi leaves

**Fig 6:** Soxhlet Extractor

Lantana (*Lantana camara* L.) plant leaves were taken as a raw material weighing 175 gm. Soxhlet extraction of these raw materials was carried out in methanol (3 cycles).Methanol was removed through rotavapour bath at 80 °C. Compound recovered was 58 gms.

Lemongrass (*Cymbopogon citrates* L.) plant leaves were taken as a raw material weighing 175 gm. Soxhlet extraction of these raw materials was carried out in methanol (3 cycles).Methanol was removed through rotavapour bath at 80 °C. Compound recovered was 55 gms.

Maddar (*Calotropis procera*) plant leaves were taken as a raw material weighing 175 gm. Soxhlet extraction of these raw materials was carried out in methanol (3 cycles).Methanol was removed through rotavapour bath at 80 °C. Compound recovered was 59 gms.

*Neem* (*Azadirachta indica*) plant leaves were taken as a raw material weighing 175 gm. Soxhlet extraction of these raw materials was carried out in methanol (3 cycles). Methanol was removed through rotavapour bath at 80 °C. Compound recovered was 58 gms.

Tulsi (*Ocimum sanctum* L.) plant leaves, flowers, branches and stem (except root) were taken as a raw material weighted 227 gm. Soxhlet extraction of these raw materials was carried out in methanol (3 cycles).Methanol was removed through rotavapour bath at 80 °C. Compound recovered was 90 ml.

Mosquito repellency test: This test provides three major parameters-a) Effective dose (ED) of the natural repellent, b) Complete protection time (CPT) provided by the repellent after application and c) Duration of its effectivity. The bioassay method is as follows- Treatment of 1%, 2% and 5% ethanolic solution of the blend of extracts. Mosquito repellency test was done by simply selecting the mosquito prone areas in the evening and night hours near the bushes in the garden, laboratory and public places to check it's effectivity. Landing and probing behavior of mosquitoes signifies the end point of the effectivity of repellent. The efficacy of the repellent of this aromatic solution against mosquitoes was done by counting numbers bitten by mosquitoes per unit time, and percentage repellencies were calculated and statistically confirmed by counting. CPT was calculated by the time elapsed by repellent application and the first mosquito bite for each dose. *Aedes aegypti* mosquito was the species which was used in the study.

**Results and Discussion**

Although this method is not fully perfect method of bioassay as several other factors influence the study of efficacy of any repellent. They may even affect the outcome and interpretation of the study. For example as far as spraying on the skin may cause some amount of absorption and evaporation of the volatiles of the extract mixture, abrasion, washing from the skin may also happen and loss could be also through perspiration, all this may cause a resultant loss in calculation of CPT. The investigations were done under laboratory conditions and human volunteers. Results of 1%, 2% and 5% are shown in Table-1.

**Table 1**

| S. No | Percentage of Blended Extract Solution | Effectivity (CPT) |
|---|---|---|
| 1. | 1% W/v | 1hour |
| 2. | 2% W/v | 2-3 hours |
| 3. | 5% W/v | 5-6 hours |

UV- visible spectra of the leaves extract of lantana, calotropis, Neem and lemon grass show presence of different molecules which have olefinic bonds as shown in figures -7, 8, 9, 10 and 11. HPLC analysis of the individual extract gives the information of major components in each extract as shown in figure- 12, 13, 14, 15 and 16.

## Conclusion

Plant-based repellents are still extensively used in this traditional way throughout rural communities in Asian sub-continent particularly in India and Srilanka because for many of the poorest communities this is the only means of protection from mosquito bites also natural smelling repellents are preferred because plants are perceived as a safe and trusted means of prevention [12, 13]. Essential oils from plants belonging to several species have been extensively tested to assess their repellent properties as a valuable natural resource particularly Citronella group [14]. Thus it was envisaged that if individually they can be effective their synergistic effect may be surely more pronounced. Thus lemon grass, lantana, calotropis, tulsi and neem were used together, the effectiveness of the blended solution was enhanced due to some non volatile components derived from calotropis, slowing down the repellency loss which is seen in volatiles mixtures if essential oils are the main component. Although the repellency of these blends at 2% w/v was comparable to that of this synthetic repellent, suggesting that further screening of different blends may lead to the discovery of more repellent combinations. Thus it can be concluded that the blended extract solution has the potential to be used as a repellent against mosquitoes very well.

These medicinal and aromatic plants can also be used for the preparation of many medicines and will have scope for other value addition products both for Indian and Srilankan markets. Technique for extraction is easy therefore it is certainly very much techno-economically viable.

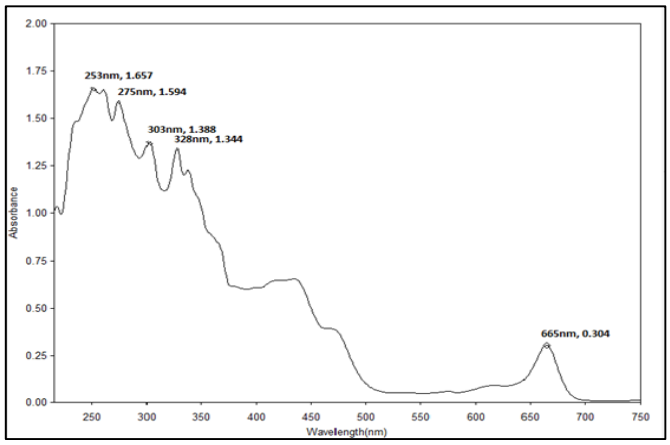


**Fig 7:** UV-Visible spectrum of Lemon grass extract

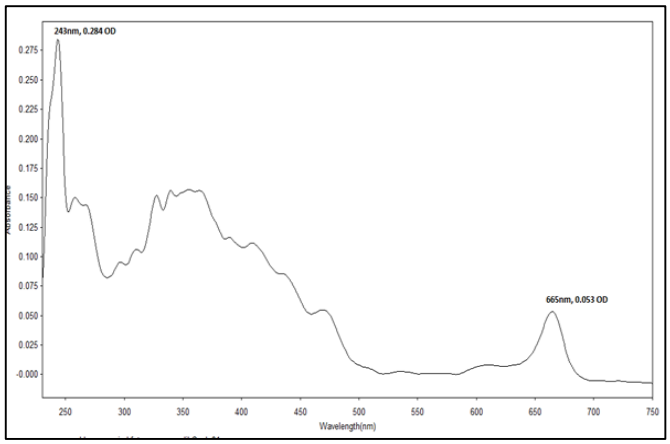


**Fig 8:** UV-Visible of Calotropis leaves extract

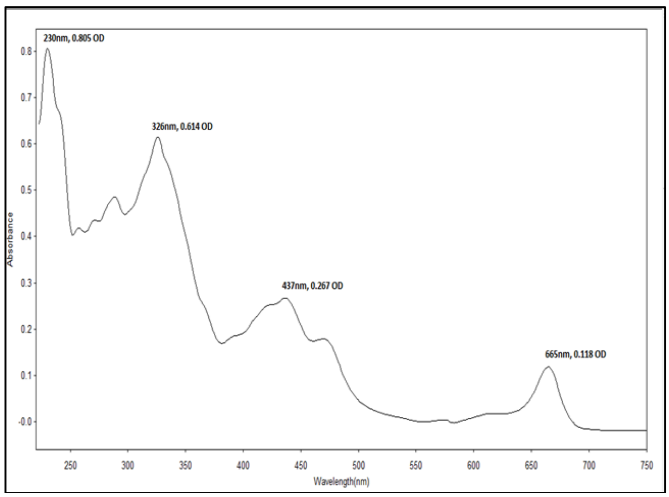


**Fig 9:** UV-Visible of Lantana leaves extract

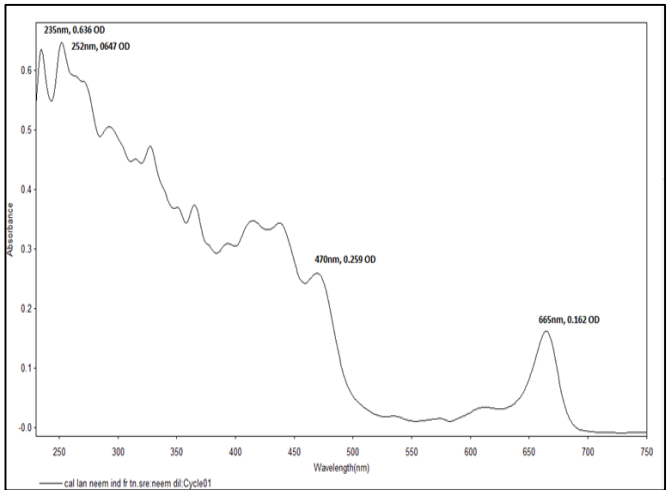


**Fig 10:** UV-Visible of Neem leaves extract

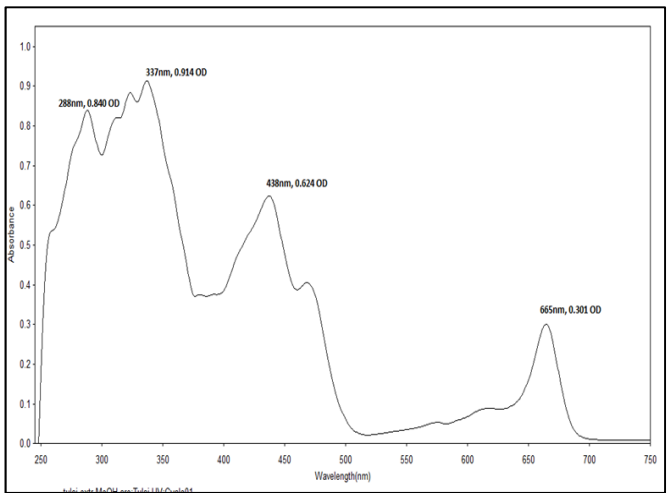


**Fig 11:** UV-Visible of Tulsi leaves extract

## HPLC of Lemon Grass

Solvent System: 80:20:0.001 MeOH:$H_2O$:O-Phosphoric Acid
Run Time: 10 min
Sample prepared in MeOH, Chromatogram taken at 255nm

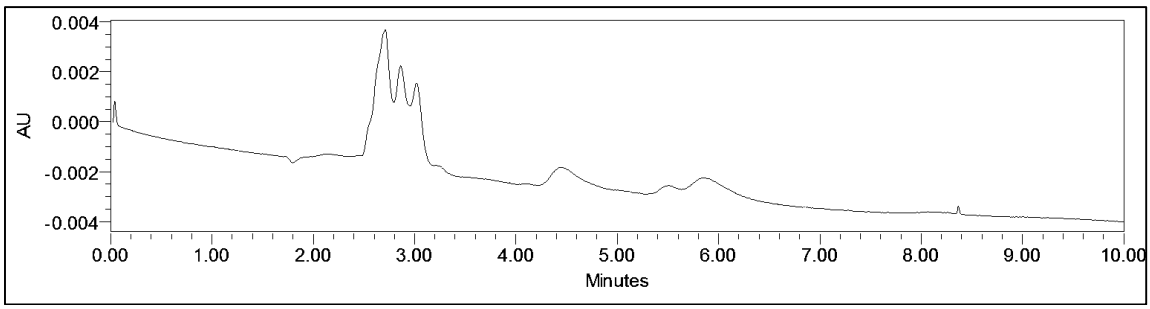


**Fig 12:** HPLC of Lemon grass leaves

**Calotropis**

Solvent System: 95:5 MeOH:$H_2O$ Run Time: 22 min Sample prepared in MeOH, Chromatogram taken on 280nm

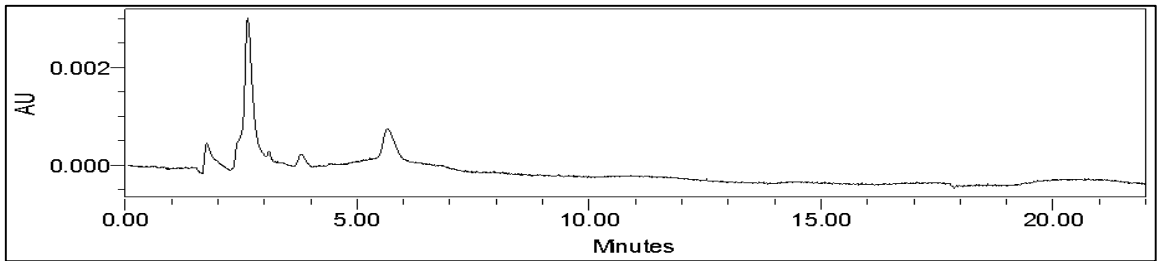


**Fig 13:** HPLC of Calotropis leaves

**Neem**

Solvent System: 95:5 MeOH:$H_2O$ Run Time: 22 min
Sample prepared in MeOH, Chromatogram taken on 280nm

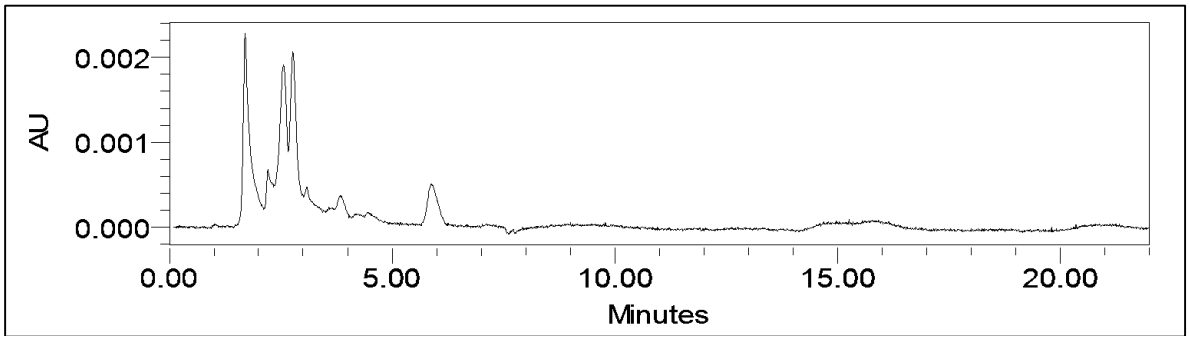


**Fig 14:** HPLC of Neem leaves

**Lantana**

Solvent System: 95:5 MeOH:$H_2O$ Run Time: 22 min
Sample prepared in MeOH, Chromatogram taken on 280nm

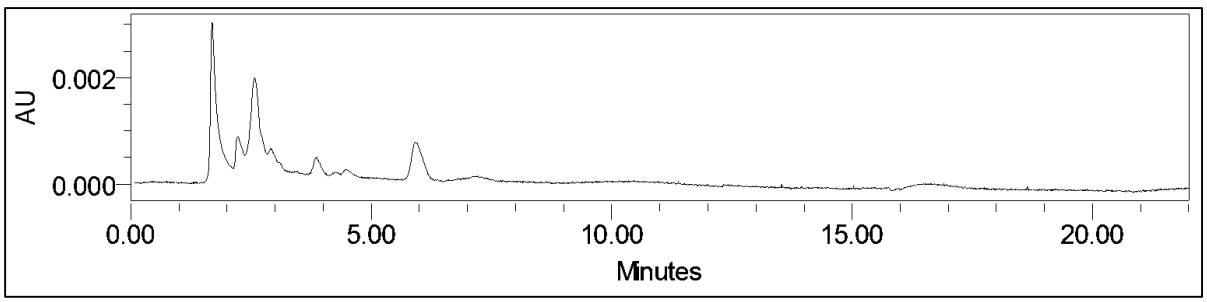


**Fig 15:** HPLC of Lantana leaves

**Tulsi**

Solvent System: 95:5 MeOH:$H_2O$ Run Time: 30 min
Sample prepared in MeOH, Chromatogram taken on 255nm

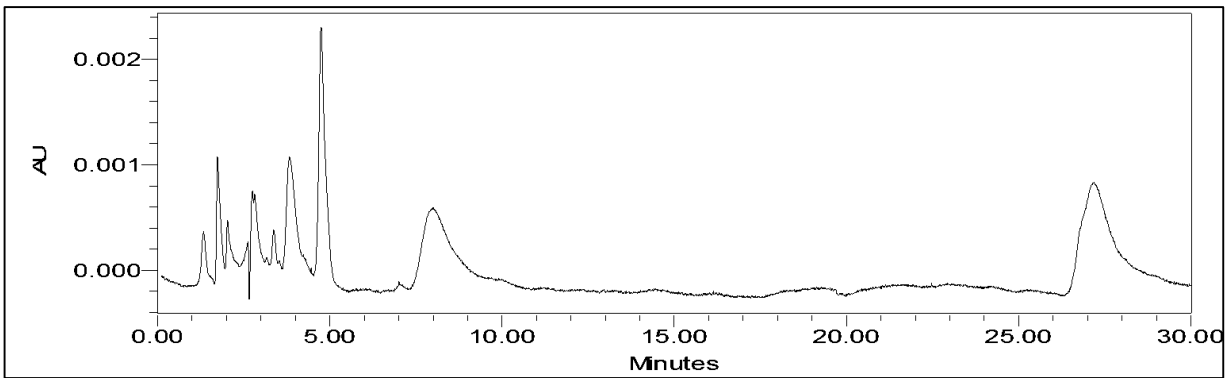


**Fig 16:** HPLC of Tulsi leaves

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