PREPARATION OF LOW COST HERBAL MOSQUITO REPELLENT FROM BEGUNIA LEAF

A Dissertation
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MASTER OF SCIENCE IN CHEMISTRY

BY
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CERTIFICATE

This is to certify that the thesis entitled **"Preparation of low cost herbal mosquito repellent from Begunia leaf"** which is being submitted by Ms. Sushree Sangeeta Pradhan to the Department of Chemistry, National Institute of Technology, Rourkela, for the award of Master of Science in Chemistry is a record of bonafied research work carried out by her under my guidance and has fulfilled the requirements for the submission of thesis, which is to my knowledge has reached requisite standard.

The results contained in this dissertation have not been submitted in part or in full to any university or institute for the award or any degree.

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CONTENTS

1. ABSTRACT

Vitex negundo (Verbenaceae) is a hardy plant, flourishing mainly in the Indian subcontinent. All parts of the plant, from root to fruit, possess a multitude of phytochemical secondary metabolites which impart an unprecedented variety of medicinal uses to the plant. Lager trials are required to prove its all activities before it is recommended in future for clinical industry. It is interesting to note that a single plant species finds use for treatment of a wide spectrum of health disorders in traditional and folk medicine; some of which have been experimentally validated. The plant is a component of a number of commercially available herbal formulations and has also shown potential as an effective bio-control agent. Employment of techniques such as cell and tissue culture would provide means of rapid propagation and conservation of the plant species and from the point of view of phytochemistry, give scope for enhancement of the quality and quantity of the bioactive secondary metabolites occurring in the plant.

Keywords: Vitex negundo; Health disorders; Mosquito repellent property; Phytochemistry.

2. INTRODUCTION

The world is gradually turning to herbal formulations which are known to be effective against a large repertoire of diseases and ailments. More importantly, they are not known to cause any notable derogatory effects and are readily available at affordable prices. However, adding a note of caution stating that plant remedies are effective and without side-effects, provided they are selected properly and taken under proper medical supervision. The active component, most often a secondary metabolite, varies in quality and quantity for a given plant species growing in different locations. The market value of such plants depends on their active content rather than merely their luxuriant growth. Plants are rich source of bioactive organic chemicals and offer an advantage over synthetic pesticides as these are less toxic, less prone to development of resistance, and easily biodegradable. Many of the herbs and shrubs are found to have promising medicinal properties, mosquito larvicidal and mosquito repellent properties. Owing to the fact that application of synthetic larvicide has envenomed the surroundings as well as non-target organisms, natural products of plant origin with insecticidal properties have been tried as an indigenous method for the control of a variety of insect pests and vectors in the recent past. Long before the advent of synthetic insecticides, plants and their derivatives were being used to kill the pests of agriculture, veterinary and public health importance. Insecticidal activity of plant-derived compounds has been evaluated and few of these exploited commercially [1]. Laboratory and field trials of plant extracts and purified chemicals showed mosquito larvicidal activity. In spite of several advancements in the field of synthetic drug chemistry and antibiotics, plants continue to be one of the major raw materials for drugs treating various ailments of humans. Clinical and pharmaceutical investigations have in fact elevated the status of medicinal plants by identifying the role of active principles present in them and elaborating on their mode of action in human and animal systems [2]. Insecticides of plant origin have been extensively used on agricultural pests, and to a very limited extent, against insect's vectors of public health importance, which deserve careful and thorough screening. The use of plant extracts for insect control has several appealing features, as these are generally more biodegradable, less hazardous, and rich storehouse of chemicals of diverse biological activity [3]. Moreover, herbal sources give a lead for discovering new insecticides. Therefore, biologically active plant materials have attracted considerable interest in mosquito control programmes in the recent times. The present

study deals with the screening of locally available herbs and shrubs for mosquito larvicidal properties. *Vitex negundo* (Family-Verbenaceae) is a hardy plant, flourishing mainly in the Indian subcontinent. All parts of the plant, from root to fruit, possess a multitude of phytochemical secondary metabolites which impart an unprecedented variety of medicinal uses to the plant. It is interesting to note that a single plant species finds use for treatment of a wide spectrum of health disorders in traditional and folk medicine; some of which have been experimentally validated [4]. The plant is a component of a number of commercially available herbal formulations and has also shown potential as an effective bio-control agent. Employment of techniques such as cell and tissue culture would provide means of rapid propagation and conservation of the plant species and, from the point of view of phytochemistry, give scope for enhancement of the quality and quantity of the bioactive secondary metabolites occurring in the plant. It belongs to kingdom plantae, order lamiales, family verbenaceae, genus *Vitex* and species *negundo.* This genus consists of 250 species of which about 14 species are found in India and some have commercial and medicinal uses. It is commonly known as Five-leaved Chaste tree or Monk's Pepper is used as medicine farily throughout the greater part of India and found mostly at warmer zones and ascending to an altitude of 1500 m in outer Western Himalayas [5]. The leaf and its extract with various solvents like alcohol, hexane, ether, acetone, chloroform etc can test for mosquito repellent properties.

2.1 Botanical description

It is a much branched aeromatic or sometimes a smaller slender tree with quadrangular, densely whitish tomentose branchlets upto 4.5 to 5.5 in height. Bark is thin, yellowish gray; leaves 3-5 foliolate; leaflets lanceolate; terminal leaflets 5-10 x 1.6-2.3 cm, lateral one smaller, all nearly glabrous. Upper surface of leaves are green and the lower surface are silvery in colour. Flower is bluish purple, black when ripe, where as root is cylindrical, long woody, tortuous with gray brown colour [Prasad and wahi, 1965]. Plant oil was effective in checking insect infestation. They show anti inflammatory, antibacterial and anti-fungal activity. It is abundant along river banks, in moist situation, open wastelands & near deciduous forests. It thrives in humid places or along water courses in wastelands and mixed open forests and has been reported to occur in Afghanistan, India, Pakistan, Sri Lanka, Thailand, Malaysia, eastern Africa. It is grown commercially as a crop in parts of Asia, Europe, North America and the West Indies [6].

3. LITERATURE

3.1 Literature study for the chemical constituents present in the plant

	Chemical constituents	Authors
Leaf	Leaves hydroxy-3,6,7,3′,4′-pentamethoxy flavone	*Banerji A. et al,* 1969 [7]
	6′-p-hydroxybenzoyl mussaenosidic acid; 2′-p-hydroxybenzoyl mussaenosidic acid	*Sehgal C. K et al,* 1986, 1983[8]
	5, 3′-dihydroxy-7,8,4′-trimethoxyflavanone; 5,3′-dihydroxy-6,7,4′- trimethoxyflavanone	*Achari B. et al,* 1984 [9]
	viridiflorol; β-caryophyllene; sabinene; 4-terpineol; gamma-terpinene; caryophyllene oxide; 1-oceten-3-ol; globulol	*Khokra S. et al* 1999 [10]
	betulinic acid [3β-hydroxylup-20-(29)-en-28-oic acid]; ursolic acid [2β –hydroxyurs-12-en-28-oic acid	*Chandramu C. et al,* 2003 [11]
	protocatechuic acid; oleanolic acid; flavonoids	*Surveswaran S, et al,* 2007 [12]
	angusid; casticin; vitamin-C; nishindine; gluco-nonitol; p-hydroxybenzoic acid; sitosterol	*Khare C.P. et al,* 2004 [13]
Essential oil of leaves	δ-guaiene; guaia-3,7-dienecaryophyllene epoxide; ethyl-hexadecenoate; α-selinene; germacren-4-ol;caryophyllene epoxide; (E)-nerolidol; β-selinene; α-cedrene; germacrene D; hexadecanoic acid; p-cymene and valencene.	*Khokra S. et al,* 2008 [14]

3.2 Some of the pharmacological activities:-

3.1.1 Analgesic activity:-

Interperitoneal administration of some leaf and root extracts using different solvents showed analgesic activity [14]. Ethanol and cold aqueous leaf extracts showed only weak effect in acidic writhing test. Whereas, chloroform and toluene leaf extracts raised the threshold of tail-flick response moderately. While studying the root extracts of plant, ethanol extract significantly increased threshold tail-flick response. In another study the methanolic leaf extract when given by I.P. route has been found to possess analgesic properties. It also potentiated Morphin and Pithidine induced analgesia significantly in dose dependent manner in mice using hot plate method as an experiment evaluated the analgesic activity of aqueous methanol leaf extract on oral administration and results showed both central and peripheral analgesic action in acitic acid writhing and tail immertion test comparable to Salysilate and Pethidine hydrochloride respectively. In rat uterus preparation, it was noticed that the inhibitory action of extract on prostaglandin biosynthesis and thereby confirming non-steroidal antiinflamatory like activity. In the other study it was also observed that leaves also contain 1.30% flavonoid compounds.

3.2.2 Anti-inflammatory activity:-

The experimental studies using various animal models have demonstrated that different parts of the plant especially leaves, fruits, roots and seeds possess anti-inflammatory and anti-arthritic activity [15]. However, possible mechanism of anti-inflammatory activity was indicated as an inhibitory action on prostaglandin biosynthesis. It is known to act by prostaglandin inhibition, may be expected to cause gastric damage but on the contrary it produces no histomorphological changes in the stomach even in toxic doses. This may be due to the selective COX-2 inhibition that might be responsible for its NSAID’s like activity. However this aspect still needs to be established.

3.2.3 Anticonvulsant activity:-

The petroleum and butanol leaf extract have shown protection, whereas, none of the root extract has shown protection against maximal electro shock seizures. Petroleum root extract could only provide protection against Leptazole induced convultions whereas, methanolic leaf extract showed significant protection against Strychnine and Leptazole induced convultions [14]. It shows not only anticonvulsant activity of ethanol leaf extract but also can potetiate the effects of

standard anticonvulsants, which may help to reduce dose and dose related side effects of standard anticonvulsants.

3.2.4 Antioxidant activity:-

This activity of the plant was studied using free radical scavenging activity effect on hydroxyl radical mediated damage to deoxyribose in vivo lipid peroxidation assay but did not show any significant effect. However, reduction of oxidative stress produced by leaf extract in albino rats has been observed. It produced significant reduction in MDA (malondialdehyde) levels after 14 days treatment in only higher dose. Although non-significant marginal rise of SOD (superoxide dismutase) in this dose was observed. In ethanol induced oxidative model, however, it significantly reduced only MDA levels in both moderate and higher doses and the effect on SOD were non-significant.

3.3 Medicinal importance of plant:-

Herbal medicine, rather than merely curing a particular disease, aims at returning the body back to its natural state of health [16]. The phytochemical components of medicinal plants often act individually, additively or synergistically in improvement of health. After having analyzed the various chemical components present in different parts of *Vitex negundo*, it is imperative that focus shifts to the medicinal applications of the plant. Myriad medicinal properties have been ascribed to this plant and the plant has also been extensively used in treatment of a plethora of ailments. These properties have been categorized under three heads – traditional medicine, folk medicine and pharmacological evidence.

3.3.1 Traditional medicine:-

Traditional medicine mainly comprises of Indian Ayurveda, Arabic Unani medicine and traditional Chinese medicine. In Asia and Latin America, populations continue to use traditional medicine as a result of historical circumstances and cultural beliefs. Traditional medicine accounts for around 40% of all health care delivered in China. Up to 80% of the population in Africa uses traditional medicine to help meet their health care needs.

a) Ayurveda:-

The plant finds mention in the verses of the *Charaka Samhita* which is unarguably the most ancient and authoritative textbook of Indian Ayurveda. *Vitex negundo* has been designated as an anthelminthic and is prescribed as a vermifuge in the exposition on the *Charaka Samhita*. Other

Ayurvedic uses of *Vn* are described by Tirtha. People sleep on pillows stuffed with *Vn* leaves to dispel catarrh and headache and smoke the leaves for relief. Crushed leaf poultice is applied to cure headaches, neck gland sores, tubercular neck swellings and sinusitis. Essential oil of the leaves is also effective in treatment of venereal diseases and other syphilitic skin disorders. A leaf decoction with *Piper nigrum* is used in catarrhal fever with heaviness of head and dull hearing. A tincture of the root-bark provides relief from irritability of bladder and rheumatism.

b) Unani medicine:-

Here the seeds are administered internally with sugarcane vinegar for removal of swellings [13]. Powdered seeds are used in spermatorrhoea and serve as an aphrodisiac when dispensed along with dry *Zingiber officinale* and milk.

c) Chinese medicine:-

The Chinese Pharmacopoeia prescribes the fruit of *Vn* in the treatment of reddened, painful, and puffy eyes; headache and arthritic joints [17].

3.3.2 Folk medicine:-

Folklore systems of medicine continue to serve a large segment of population, especially those in rural and tribal areas, regardless of the advent of modern medicine [18]. The entries regarding the multifarious applications of *Vn* in folk medicine have been grouped regionally to emphasize the ethnobotanical diversity and ubiquity of the plant; and the details have been laid out in Table 1 and 2.

Table – 1

Sl.No	COUNTRY	USED IN TREATMENT OF
1	Bangladesh	Weakness, Headache, Vomiting, Malaria, Black fever[19]
2	China	Common cold, Flu and Cough[20]
3	Nepal	Sinusitis, Whooping cough[21]
4	Pakisthan	Chest-pain, Backache, Used as toothbrush, Used as anti-allergenic agent, Gum and skin diseases, Used as medicine for buffaloes in colic[22], [23], [24]
5	Philippines	Cancer[25]
6	Srilanka	Eye disease, Toothache, Rheumatism, Used as a tonic, carminative and vermifuge[26]

Table – 2

S. No.	STATE	USED IN TREATMENT OF
1	Andhra Pradesh	Asthma, Cancer, Used as bath for women in puerperal state and for new born children[27]
2	Assam	Jaundice, Urticaria, Cellulitis, Abcesses, Carbuncles, Eczema, Liver disorders[28]
3	Himachal Pradesh	Kwashiorkor, Wounds, Body ache
4	Karnataka	Toothache, Febrile, catarrhal and rheumatic afflictions Migraine[29]
5	Maharashtra	Rheumatism, Encephalitis, Expectorant, Joint pain
6	Orissa	Jaundice
7	Tamil Nadu	Used as antidote for snake bite, Respiratory disorders, Fever, Headache
8	Uttar Pradesh	Eye pain, Used as refrigerant for cattle, 48 types of ailments[30]

Pharmacological evidence:-

Demands of the scientific community have necessitated experimental evidence to further underline the medicinal importance of *Vn* described above. Taking cue from these traditional and folk systems of medicine, scientific studies have been designed and conducted in order to pharmacologically validate these claims.

a) Histomorphological and cytotoxic effects:-

The histomorphological effect of *Vn* extracts in rats was studied and it was found that the stomach tissue were unaffected even by toxic doses; while dose-dependent changes were observed in the heart, liver and lung tissues. Cytotoxic effect of leaf extracts of *Vn* was tested and affirmed using COLO-320 tumour cells. On one hand, it was found that the chloroform extracts of *Vn* leaves were toxic to a human cancer cell line panel while on the other hand, this *Vn* extracts were non-cytotoxic on mammary and genito-urinary cells of mice [31].

b) Effect on reproductive potential

The flavonoid rich fraction of seeds of *Vn* caused disruption of the latter stages of spermatogenesis in dogs and interfered with male reproductive function in rats. It must however be noted that these findings are in sharp contrast with the traditional use of *Vn* as aphrodisiac. The ethanolic extracts of *Vn* showed estrogen-like activity and propounded its use in hormone replacement therapy [32].

4. OBJECTIVE

- Extractions of leaf were done by soxhlet extractor.
- Then separation of oil was done from the extract by vacuum evaporator.
- Determination of chemical constituents present in oil and hexane extract.
- To find out the mosquito repellent properties of the extract.
- Preparation of mosquito repellent from the extract and then leaf.
- Then to find the constituents of extract responsible for mosquito repellent properties.
- Other uses of leaf.

5. EXPERIMENTAL

5.1 Extraction of leaf:-

- Solvent extractions of leaf were done by using different solvents like Ethyl alcohol, Diethyl ether and Hexane.
- Then one part of the extracts obtained were concentrated under reduced pressure in vacuum evaporator at (40 – 50)°C into a syrupy liquid.
- This syrupy liquid was again separated several times in separating funnel to get the desire oil. The constituents of the oil were determined by GCMS.
- The constituents of the hexane extract were also determined by GCMS.
- The other part of the extracts was used for the preparation of mosquito repellent liquid.

5.2 Preparation of herbal mosquito repellent from leaf and extract:-

5.2.1 From Leaf:-

- The leaves were first collected and air dried. Then 500 gm of leaves were powdered and mixed with powder of jhuna, saw dust and cow dung in different proportion which was subsequently moulded to form agarbati.

5.2.2 From Extracts :-

- The extract obtained from Ethyl alcol, Diethyl ether and Hexane were kept separately and the extracts were concentrated by evaporating the solvent.
- Then each extract were mixed together in 1:1:1 ratio to form a mixture.

5.3 Calculation of mortality rate of mosquito :-

Calculation result

Henderson-Tilton's formula

$$\text{Corrected \%} = \left(1 - \frac{\text{n in Co before treatment} * \text{n in T after treatment}}{\text{n in Co after treatment} * \text{n in T before treatment}}\right) \text{ X 100}$$

Where : n = Insect population, T = treated, Co = control

Abbott formula

$$\text{Corrected \%} = \left(1 - \frac{\text{n in T after treatment}}{\text{n in Co after treatment}}\right) \text{ X } 100$$

Where : n = Insect population, T = treated, Co = control

5.4 Charactersation:-

- The CHNS analysis and the IR spectra of Diethyl ether, Hexane, Ethanol and Mosquito repellent liquid have been done.
- Also the chemical constituents present in oil and hexane extract were determined by GCMS.

6. RESULTS AND DISCUSSION

6.1 CHNS Analysis:-

	N%	**C%**	**H%**	**S%**
Hexane Extract	1.462293	1.027159	0.151977	0.281124
Ether Extract	0	20.42419	2.776406	0.825022
Alcohol Extract	0	1.347028	0.194001	0.371852
Mosquito repellent liquid	0.358947	107.668	15.53009	0.143442

- The CHNS analysis of the hexane, ether and alcohol extracts and also the mosquito `repellent liquid has been done.
- It was found that the N%, C%, H% and S% in hexane extract is 1.462293, 1.027159, 0.151977, and 0.281124 respectively.
- The ether extract contains 0% of N, 20.42419% of C, 2.776406% of H and 0.825022% of S. The alcohol extract contains 0% of N, 1.347028% of C, 0.194001% of H and 0.371852% of S.
- Similarly the mosquito repellent liquid contains 0.358947% of C, 107.668% of N, 15.53009% of H and 0.143442% of S.

6.2 GCMS Analysis of hexane extract and oil

6.2.1 Chemical constituents of hexane extract

CONSTITUENTS	PERCENTAGE
Viridiflorol	19.55
β-caryophyllene	16.59
Sabinene	12.07
4-terpineol	9.65
γ-terpinene	2.21
Caryophyllene	1.75
1-ocetene-3-ol	1.59
Globulol	1.05
Rest of the extract	35.54

6.2.2 Chemical constituents of oil:-

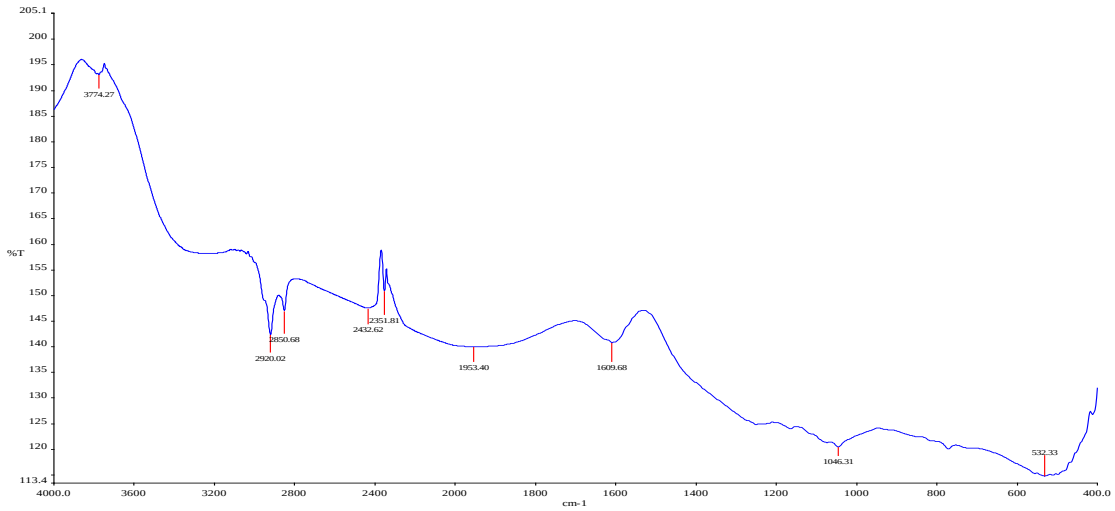
CONSTITUENTS	PERCENTAGE
Pinene	11.52
Limonene	9.26
Camphene	9.24
Phellandrene	6.19
Methylheptanone	9.03
Cumene	8.61
Linalool	7.56
Camphor	8.12
4-terpineol	8.01
Citral	5.32
Caryophylline oxide	2.26
Caryophyllene	2.12
Terpinyl acetate	1.56
Rest of the constituents	10.7

This is the percentage of chemical constituents present in hexane extract which was determined by GCMS. Here the hexane extract contains viridiflorol 19.55%, β-caryophyllene 16.59%, Sabinene 12.07%, 4-terpineol 9.65%, γ-terpinene 2.21%, caryophyllene 1.75%, 1-ocetene-3-ol 1.59%, globulol 1.05%. The rest 35.54% present in rest of the extract.

Similarly the percentage of chemical constituents present in oil was also determined by GCMS. The oil contains 11.51% pinene, 9.26% limonene, 9.24% camphene, 6.19% phellandrene, 9.03% methylheptanone, 8.61% cumene, 7.56% linalool, 8.12% camphor, 8.01% 4-terpineol, 5.32% citral, 2.26% caryophylline oxide, 2.12% caryophylline, 1.56% terpinyl acetate. Rest of the 10.7% contains rest of the constituents.

6.4 FTIR study

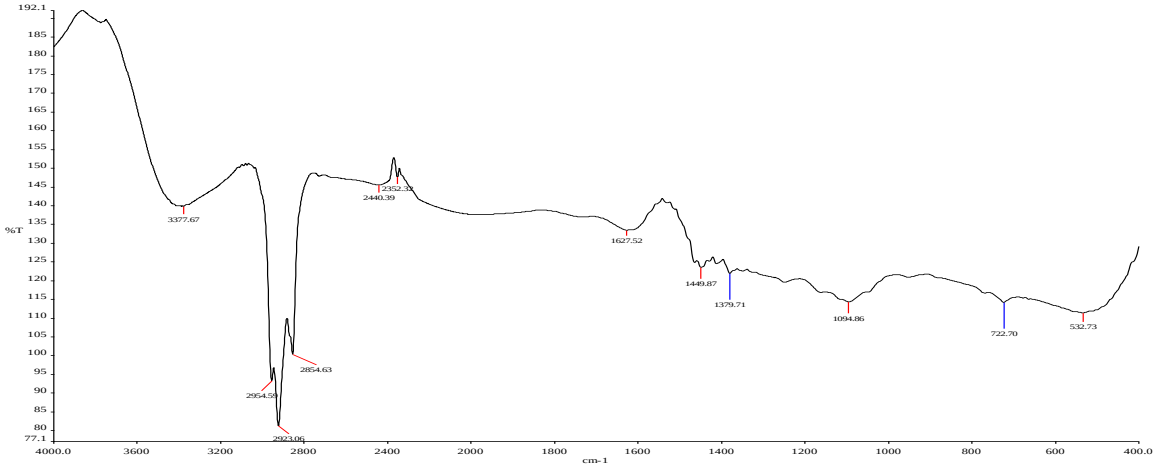
6.4.1 FTIR Spectra of alcohol extract:-



(Fig.1)

The peak at around 2920.02 and 2850.68 indicates the presence of O-H bond of strong intensity of carboxylic acid and derivative group. The peak at around 1609.68 indicates the presence of C=C (alkenes) bond of medium intensity. The peak at around 1046.31 indicates the presence of S=O (sulfoxide) bond of strong intensity of group. The peak at around 532.33 indicates the presence of S-S bond of weak intensity. The peak at around 1953.40 indicates the presence of C=C (alkenes) bond of strong intensity.

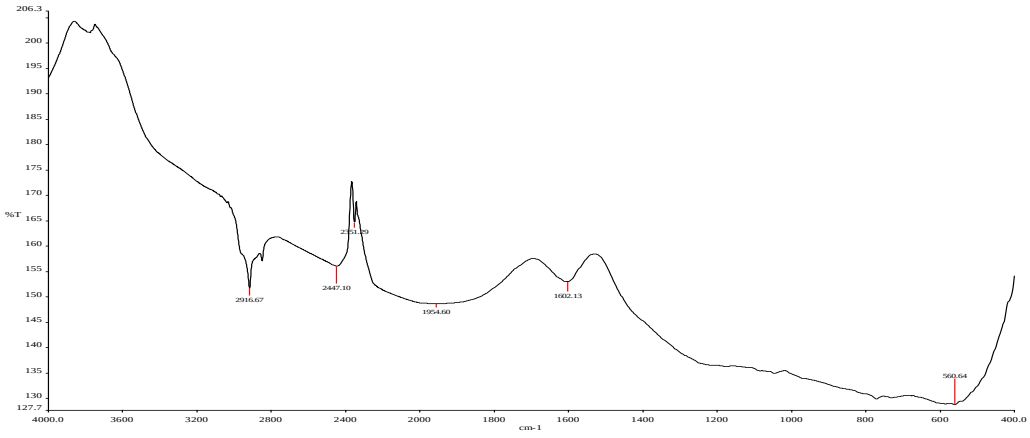
6.4.2 FTIR Spectra of ether extract



(Fig.2)

The peak at 3377.67 indicates the presence of N-H (secondary amine) bond of weak intensity. The peak at 2954.59, 2923.06, and 2854.63 indicates the presence of O-H bond of strong intensity of carboxylic acid derivative group. The peak at around 1627.52 indicates the presence of strong intensity of amide group. The peak at around 1094.86 indicates the presence of Si-OR bond of strong intensity. The peak at around 722.70 indicates the presence of S-OR bond of strong intensity.

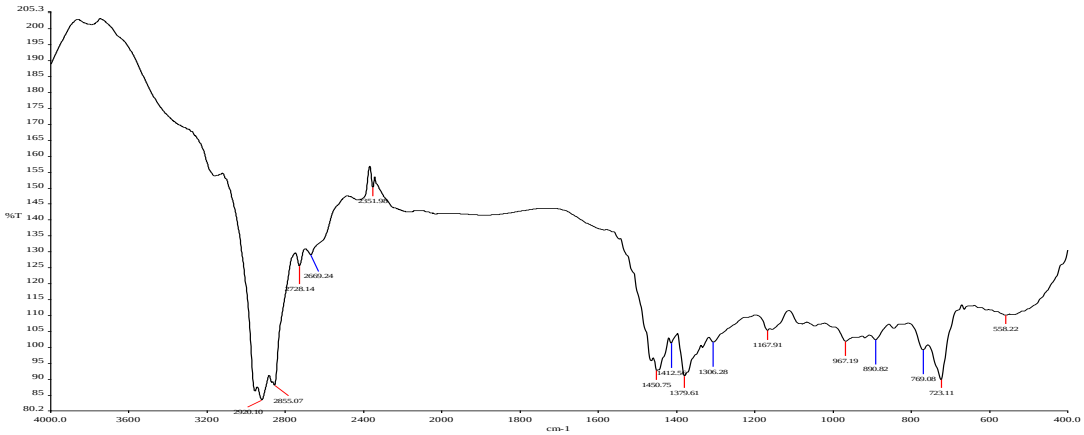
6.4.3 FTIR Spectra of hexane extract



(Fig.3)

The peak at around 2916.67 indicates the presence of O-H bond of strong intensity of carboxylic acid and derivative group. The peak at around 1602.13 indicates the presence of NH_2 (primary amine) of medium to strong intensity.

6.4.4 FTIR Spectra of mosquito repellent



(Fig.4)

The peak at around 2920.10, 2855.07, 2728.14, and 2669.24 indicates the presence of O-H bond of strong intensity of carboxylic acid and derivative group. The peak at 1375.61 and 1412.55 indicates the presence of CH_3 bending of strong intensity. The peak at 1167.91 indicates the presence of P=O (Phosphin oxide) of strong intensity. The peak at 967.19 indicates the presence of P=OR (ester group) of strong intensity. The peak at 890.82 indicates the presence of S=OR group of strong frequency.

7. CONCLUSIONS

- Vitex negundo represents a class of herbal drug with very strong conceptual or traditional base as well as strong experimental base for its use.
- Thus this plant has great potential to be developed as a drug by pharmaceutical industries.
- The essential oil as well as powder is having excellent mosquito repellent properties.
- Partial analyses of the constituents are done.
- Insect repellent properties of the leaf are also proved.

8. FUTURE WORK

- The mixture is acting as moquito repellent but to increase its effectiveness,the actual constituent of the extract is to be identified and steps can be taken to synthesize the compound.
- To find the toxicity of the extract.
- The toxicity test can be conducted only when the composition and constituents are known**.**

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