EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 1
Issue: 3
306-325
Year: 2014

ACTIVITY OF SELECTIVE DIFFERENT OILS AGAINST PEDICULUS HUMANUS CAPITIS

Ms. Trupti D. Dhumal, Dr. J.S. Waghmare*

Department of Oils, Oleo chemicals and Surfactants Technology Institute of Chemical Technology, Mumbai-400019.

Article Received on 02/10/2014 **Article Revised on 25/10/2014** **Article Accepted on 18/11/2014**

***Correspondence for Author**
Dr. J.S. Waghmare
Department of Oils, Oleo chemicals and Surfactants Technology Institute of Chemical Technology, Mumbai-400019.

ABSTRACT

Head lice infestation is an emerging social problem in undeveloped and developed countries. Because of louse resistance increasing, several long-used insecticidal compounds have lost their efficacy, and alternatives, such as essential oils, have been proposed to treat this parasitic infestation. Non-toxic alternative options are hence needed for head lice treatment or prevention and natural products from plants, especially essential oils (EOs) are good for safer control agents that may provide good anti-lice activity and low levels of evolved resistance and also other non-edible oil that have head lice resistance activity. A few Essential oils have been tested as repellents with promissory results, although often in vitro tests and clinical trials produce contradictory results. The use of pyrethroids to control head louse infestations have suffered considerable loss of efficacy due to the development of resistance. In the last past few years, several new alternative products to synthetic pyrethroids have been developed and are sold in the market against head lice. The present study investigated the efficacy of some essential oil and non-edible oil that have high medicinal value and therefore use against head lice as Chemical constituents of these oils present a wide range of biological activities. The aim of the present research study was to investigate the pediculicidal activity of essential oils and non-edible oil that have high medicinal value like clove and eucalyptus oil as essential, neem oil as non-edible and also camphor in coconut oil and compare the relative toxicity. The oil was obtained by hydro-distillation and by solvent extraction process and their analysis done through use of FTIR and GC. A filter paper diffusion bioassay method was carried out in order to determine the pediculicidal activity of oils. The pediculicidal potential of different

concentration of oils of clove, eucalyptus, neem and camphor in coconut oil (Camphorated oil) was found to be excellent for head lice and the mean death time observed with high active clove oil was overall as 24.49 minutes, which was comparable to other group oil.

KEYWORD: insect repellents, contradictory *India.*

1. INTRODUCTION

The 3 major lice that infest humans are:

1. Pediculus humanus capitis (head louse),
2. Pthirus pubis (crab louse) and
3. Pediculus humanus humanus (body louse).

Patients with louse infestation present with scalp pruritus, excoriations, cervical lymphadenopathy and conjunctivitis. A hypersensitivity rash also results from it. Head lice infestation crosses all economic and social boundaries and therefore, Lice infestation of any part of the body is known as “Pediculosis”.

Head lice or louse are tiny wingless parasites biologically known as pediculus humanus capitis that inhabit and thrive on hair and the scalp. They feed on very small amount of blood that they draw from the scalp. Head lice infestation is common in all over among children 3 to 12 years of age approximately 4 to 10 million have infestations each year. Head lice are not a health hazard or a sign of uncleanliness and are not responsible for the spread of any disease. The most common symptom is itching. Individuals with head lice infestation may scratch the scalp to alleviate itching and there rarely may be secondary bacterial skin infection. Head lice are the cause of much embarrassment and misunderstanding, many unnecessary days lost from school and work as millions of dollars spent on remedies.

Many Dermatologists says that, "Head lice problem occurs more in women than men, because women usually have longer hair. Loose long hair is more susceptible to lice. And managing a lice infestation is more difficult on a long-haired person, as it is difficult to comb, inspect and treat." Head lice are passed from person to person by direct contact with the hair of an infected person. Cosmetic dermatologist and trichologist says that, "Anyone who comes in close contact with someone who already has head lice or even their contaminated clothing and other belongings such as hats/caps, scarves, coats, sports uniforms or hair ribbons is at risk of an infestation too." Personal contact is common during play and sports activities and

at school/college, home, slumber parties or camps amongst children and teenagers. One should refrain from using infested combs, brushes or towels and avoid lying on a bed, couch, pillow, carpet or keep away from stuffed animals that has recently been in contact with a person with lice.

But actually Trichologist says that, "Lice aren't dangerous and don't spread any particular disorder but are contagious and cause itching that can be terribly annoying and embarrassing. Lice bite may cause one's scalp to become itchy and inflamed and persistent scratching may lead to skin irritation and even infection. It can lead to a bacterial infection which causes the skin to become red and tender and also involves crusting and oozing of pus along with swollen lymph glands."

Symptoms

- ✓ Intense itching of the scalp.
- ✓ Small, red bumps on the scalp, neck, and shoulders (bumps may become crusty and ooze).
- ✓ Tiny white specks (eggs, or nits) on the bottom of each hair those are hard to get off.

The control of human head lice worldwide depends primarily on the continued applications of organochlorine (DDT and lindane), organophosphorus (malathion), carbamate (carbaryl), pyrethrin, pyrethroid (permethrin and 6-phenothrin) and avermectin (ivermectin-originated from Streptomyces avermitilis) insecticides.[1, 2, 3] The repeated use of permethrin and other insecticides for the control of head lice during past decades has resulted in the development of marked levels of resistance. Thus, new alternative insecticides are needed for the control of head lice. We studied the fumigant and repellent properties of individual essential oils and non-edible oil against head lice. Many modern pediculicides tend to fail because of low efficacy on lice eggs, whereas essential oil constituents are reputed to have good ovicidal capabilities[4]. They are responsible for the characteristic odors of plants such as eucalyptus, pine, mint, peppermint, and lemon. Several plant products such as aniseed, coconut, neem and tea tree oils are used in different available compositions for the treatment of head lice infestation.

So, the present study was done to explore the licicidal potential to investigate a killing effect on lice with the formulation containing essential oil and non-edible oil. It was also

determined that whether a minimum concentartion could suffice to give a licicidal effect, or whether increase in concentration contration would perform significantly better.

Essential oil

Essential oils are very complex mixtures which can contain about 20–60 components at quite different concentrations. They are characterized by two or three major components at fairly high concentrations (20–70%) compared to others components present in trace amounts. For example,

1. In Origanum compactum essential oil, carvacrol (30%) and thymol (27%) are the major components,
2. Linalool (68%) of the Coriandrum sativum essential oil,
3. 1, 8-cineole (50%) of the Cinnamomum camphora essential oil.

Generally, these major components determine the biological properties of the essential oils. The components include two groups of distinct biosynthetical origin, [7, 8, 9, 10]

A. Clove oil

Clove oil uses date back to ancient China and India. Chinese used clove for treatment of hernia, diarrhea and bronchitis. Traditionally clove was used for intestinal parasites, skin infections, digestive upsets and toothaches. Today, it is still known for its anti-infectious, analgesic and anti-inflammatory properties. This is oil is antiseptic and a very strong antioxidant. *Clove oil* is one of the strongest natural *antioxidants* ever tested. It can increase blood circulation, which makes it one of the best supports for hair growth stimulation[11].

B. Eucalyptus oil

Cineole-based eucalyptus oil is used as an insect repellent and biopesticide. In the U.S., eucalyptus oil was first registered in 1948 as an insecticide and miticide[12]. Eucalyptus oil has a history of wide application, as a pharmaceutical, antiseptic, repellent, flavouring, fragrance and industrial uses. The leaves of selected Eucalyptus species are steam distilled to extract eucalyptus oil.

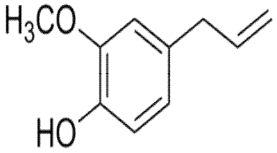
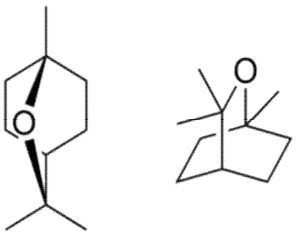
S. No	Essential oil	Constituents	Major Constituent
1.	Clove Bud	Eugenol, Eugenol Acetate, Iso-Eugenol And Caryophyllene.	Eugenol 
2.	Eucalyptus	1, 8-Cineole, α -Pinene, β -Pinene, α -Phellandrene, Limonene, Terpinen-4-Ol, Aromadendrene, Epiglobulol, Piperitone And Globulol.	1, 8-Cineole 

Table1: Essential oil and their main constituents.

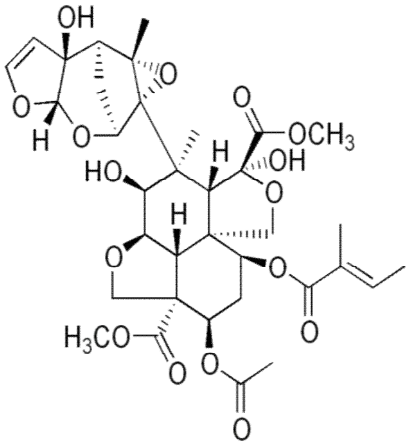
OTHER OIL

A. Neem oil: Neem oil is a vegetable oil obtained from Azadirachta indica, an evergreen tree native to India. Extracts from the fruits and seeds of the plant yield a light orange to dark brown, thick oily substance that has a very pungent odor. It is not soluble in water. The active ingredient of neem oil against lice appears to be azadirachtin, an organic tetranortriterpenoid molecule similar to an insect molting hormone, which disrupts the insect life cycle. Other components such as steroids and triterpenoids are also part of neem oil composition. [13]

B. Coconut Oil and Camphor

Camphorated oil (50% camphor in coconut oil) was strengthening the weak roots and promotes hair growth. Due to the substances that encourage blood circulation, camphor nourishes the hair follicles and alleviates thinning hair by restoring the gloss and sheen of the strands. The camphorated coconut oil form by boiling of camphor bits in coconut oil and their mixture directly use against head lice as the camphorated coconut oil suffocates the head lice.

Table2: Neem oil constituents.

S. No	Oil	Constituents	Major Constituent
1.	Neem oil	Azadirachtin, Nimbin, Nimbidin, Nimbidol, Sodium-nimbinate, Gedunin, Salannin, Quercetin.	Azadirachtin 

4. MATERIALS AND METHODS

2.1 Experimental organisms

Head lice were collected from children of 6-13 years old, using a fine toothed comb. Lice were obtained from slum area, Mumbai, where a topical method indicated high resistance levels to permethrin. After collection, head lice were placed in an environmental chamber at 18 ± 0.5ºC and 70-80% RH in darkness.

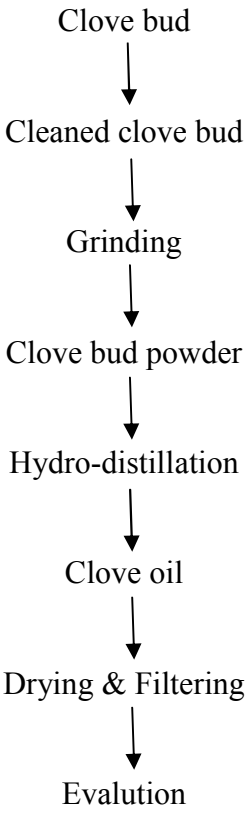
2.2 Plant material

The raw material clove, eucalyptus and neem consist of buds, leaves and seeds. The clove bud and neem seed purchased from the local market and eucalyptus leaves obtained from Institute of chemical technology, Mumbai. The material was naturally dried in shadow and stored in controlled laboratory conditions. For camphor in coconut oil, Coconut oil and camphor bits purchased from local market.

2.3 EXTRACTION OF OILS

2.3.1 Isolation of Essential oil

25gm of clove bud were hydro distilled with 250ml of distilled water in Clevenger type apparatus without organic solvent for 5-6hr. The essential oil was dried over anhydrous Na_2SO_4 and stored in dark color glass bottle.

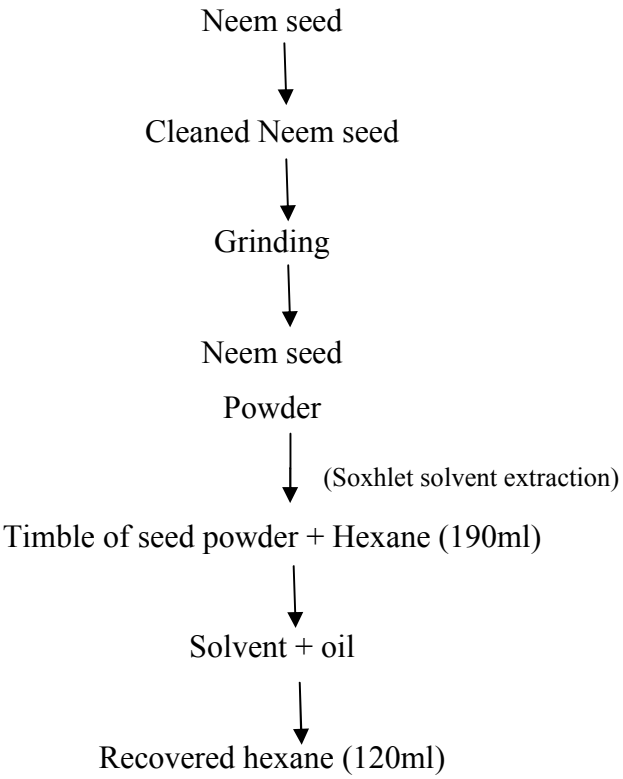


The oil obtained through hydro-distillation gives rise to about 13.86% yield of clove oil and for eucalyptus oil, eucalyptus leaves collected and dried then in same way of clove oil hydro-distillation procedure, the eucalyptus oil was obtained. The yield obtained for 30gm of leaves was 3.04%.

2.3.2 Isolation of Neem oil

The extraction of neem oil carried out by using solvent extraction process with use of solvent like Hexane.

This was carried out in a 250 ml Soxhlet apparatus on a heating mantle. The neem seed powder was packed inside a muslin cloth placed in a thimble of Soxhlet extractor. A round bottom flask containing Hexane was fixed to the end of the extractor and a condenser was tightly fixed at the bottom end of the extractor. The flask was heated at 60ºC with the use of an electric mantle. The solvent then vaporized and condensed into the evaporator. The mixture obtained (solvent and oil) moved directly into a round bottom flask. The process continues for the specified time. Oil was recovered by distillation process using the rota-evaporter apparatus. The oil obtained was stored in a bottle for further processes.



3. Physical characterization of oil

3.1 Refractive index [14]: The abbe's refractometer was used for the measurement of refractive index. To achieve accuracy apparatus should be calibrated against distilled water, which has refractive index of 1.3325 at 25°C. After calibration samples refractive index was measured.

3.2 Specific gravity or weight per milliliter [14]

Weight per milligram of a liquid is weight in gram of 1 ml of a liquid when weighed in air at 25°, unless otherwise specified.

Procedure: Thoroughly clean and dry pycnometer was selected. Specific gravity of liquid was obtained by dividing the weight of liquid contained in the pycnometer by the weight of water contained, both determined at 25°C.

3.3 Moisture

Moisture content of all the samples were carried out by constant oven drying method.

3.4 Phytochemical screening: Phytochemical screenings were performed using standard procedures. [14, 15]

3.4.1. Test for reducing sugars (Fehling's test)

The aqueous ethanol extract (0.5 g in 5 ml of water) was added to boiling Fehling's solution (A and B) in a test tube. The solution was observed for a colour reaction.

3.4.2. Test for anthraquinones

0.5 g of the extract was boiled with 10 ml of sulphuric acid and filtered while hot. The filtrate was shaken with 5 ml of chloroform. The chloroform layer was pipette into another test tube and 1 ml of dilute NH_3 was added. The resulting solution was observed for colour changes.

3.4.3. Test for terpenoids (Salkowski test)

To 0.5 g each of the extract was added 2 ml of chloroform. 3ml of concentrated H_2S0_4 was carefully added to form a layer. A reddish brown colouration of the interface indicates the presence of terpenoids.

3.4.4. Test for flavonoids

5ml of dilute ammonia was added to a portion of an aqueous filtrate of the extract.

Concentrated sulphuric acid (1 ml) was added. A yellow colouration that disappear on standing indicates the presence of flavonoids.

3.4.5. Test for tannins

About 0.5 g of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

3.4.6. Test for alkaloids

0.5 g of extract was diluted to 10 ml with acid alcohol, boiled and filtered. To 5 ml of the filtrate was added 2 ml of dilute ammonia. 5 ml of chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 ml of acetic acid. This was divided into two portions. Mayer's reagent was added to one portion and Draggendorff's reagent to the other. The formation of a cream (with Mayer's 0reagent) or reddish brown precipitate (with Draggendorff's reagent) was regarded as positive for the presence of alkaloids.

3.4.7. Test for cardiac glycosides (Keller-Killiani test)

To 0.5 g of extract diluted to 5 ml in water was added 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric

acid. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.

4. Chemical characterization of oil

4.1 Fourier Transform Infrared Spectroscopy (FTIR)

In order to determine the functional groups present in the oil, Fourier Transform Infrared spectroscopy was used.

It related with qualitative and quantitative determinations of different components present in the biomaterials. Infrared Spectra were recorded in a spectrophotometer shimadzu FTIR model happ-genzel in a frequency range from 4500 cm^{-1} to 500 cm^{-1}. The specimens with exposure area of 1 cm^2 were prepared as it mentioned above. The liquid sample was directly place on platform and sampling was done. Before and after sampling the specimen were clean by using n- hexane and again washed with distilled water and then dried. Subsequently, part of the surface of material was corresponding to obtain the infrared spectrum of specimen.

4.2 Gas Chromatography

GC analyses were performed using an Aglient GC- 2010 gas chromatograph equipped with a FID and capillary column (325 C 30 m X 320 mcm X 0.25 mcm). Oven temperature was 60^{o}C for 2 min then programmed heating from 60 to 150^{o}C at a rate of 10^{o}C/min, and at 220^o C for inlet temperature. Injector and detector temperatures were 250^{o}C. The carrier gas nitrogen was adjusted to a linear velocity of 24 ml/min. The samples were injected into the GC by split mode with a split ratio.

5. PREPARATION OF PEDICULICIDAL FORMULATIONS ANS BIOASSAY

5.1 Preparation of Oil of different concentration for pediculicidal activity

Based on the concentration found in literatures, total four different group of individual oil concentration were prepared as 0.0625, 0.125, 0.25, 0.50 and 0.75 mg of oil in 80µl of acetone for pediculicidal activity. These all oils were investigated for their licicidal activity against Human head lice in various concentrations by filter paper diffusion bioassay[15] (Table7).

5.2 Methodology

The in-vitro tests were started within 1 hr after collection of lice. A filter paper diffusion bioassay was made[15]. After careful selection of lice, a filter paper discs (Whatman No.1, 2 cm in diameter) coinciding with internal diameter of petri dish were cut and placed in petridishes. Petridishes containing filter papers impregnated with the formulations and lice were covered with glass lids and incubated under normal maintenance conditions for lice of 28±2 °C, 60±20% relative humidity (RH).

5.3 Bioassay

A filter paper contact bioassay was used to evaluate the toxicity of the essential oils, non-edible oil and insecticides to P. humanus capitis. In a preliminary experiment with 0.0625 mg/cm^2 dose was an appropriate starting dose for a primary screening. If an essential oil gave better activity then further bioassays were conducted[6]. Amounts 0.0625, 0.125 and 0.25 mg/cm^2 of each oil were applied to filter papers (Whatman No.1, 2 cm in diameter) in 80 µl of acetone. Control filter papers received 80µl of acetone. After drying in a fume hood for 2 min, each filter paper was placed on the bottom of a petri dish (4.5 cm in diameter). Batches of 4-6 P. humanus capitis, were placed on each petri dish, containing a few strands of human hair, and the dish covered with a lid. Treated and control (solvent only) lice were held at 31^{o}C and 65 5% RH in darkness. Mortalities were determined every 5 min for 5 h. lice were considered dead if they exhibited lethargic response or no movement.

The lice were judged as dead if there were no vital signs such as movements of antennae or minimal leg movements (with or without stimulation by a forceps). The petridish lids were kept in place during the tests but removed every 15 min so that the lice could be observed and the number of fatalities recorded. Death was defined as lack of movement of limbs and gut, and failure to respond when the legs were stroked with forceps. [16, 17]

6. RESULTS AND DISCUSSION

6.1 PHYSICAL PROPERTIES OF OIL

In the table below are shown some of the physical properties of Clove oil, Eucalyptus oil, Neem oil and Camphorated coconut oil such state, color, odor, refractive index, specific gravity, solubility, moisture content and oil yield percentage.

Table3: Properties of oil.

Properties↓ /oils→	Clove oil	Eucalyptus oil	Neem oil	C.Camphorated oil
State	Liquid	Liquid	Liquid	Liquid
Color	Colorless-light yellow	Colorless	Brown	Colorless
Odour	spicy	Aromatic	Garlic	Charectistics
R.I	1.5272	1.4564	1.4462	--
Sp. Gravity	1.045	0.920	0.9137	0.9224
Solubility	Methanol and Diethyl ether	Slightly in methanol	Slightly in methanol	Ethanol
Moisture content of raw	16.12%	12.73%	15.81%	--
% oil(raw)	13.86%(25)	3.48%(20)	35.87%(3)	--

It is observed that, specific gravity of neem, eucalyptus and camphorated coconut oil is less that water and clove oil and this shows that all oil have good flow ability with less viscous characteristics. Almost all oil shows organic solvent solubility as mainly in methanol, ethanol and diethyl ether.

6.2 Phytochemical screening of plant materials

The phytochemical screening of the essential oil studied showed the presence of reducing sugar and terpenoids (Table 4). Clove oil, eucalyptus oil, neem oil and camphorated coconut oil showed the absence of anthraquinones, tannins and alkaloids. Clove oil tested negative for the presence of alkaloids, cardiac glycosides and only eucalyptus oil tested negative for the presence of flavonoids.

All the plants exhibited potent antioxidant activity. The presence of flavonoids in the plants is likely to be responsible for the free radical scavenging effects observed. Flavonoids is phenolic compounds and plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers. [18] that have maximum synergetic activity.

Table4: Phytochemical results.

S. No	Test /oils	Clove	Eucalyptus	Neem	Camphorated coconut oil
1.	Reducing sugar	√	√	√	√
2.	Anthroquinones	--	--	--	--
3.	Terpenoids	√	√	√	√
4.	Flavonoids	√	--	√	√
5.	Tannins	--	--	--	--
6.	Alkaloids	--	--	--	--
7.	Cardiac glycosides	--	√	--	--

6.3. Chemical characterization of oil

6.3.1 FOURIER TRANSFORM INFRARED SPECTROSCOPY (FT-IR)

FT-IR is used to determine the different functional group such as phenol, alcohol, alkane, alkynes, alkyl halide, alkenes and other such groups present in the substance which here is clove oil, eucalyptus oil, neem oil and camphorated coconut oil.

Table5: FTIR Frequency range obtained for clove oil.

Frequency cm^{-1}	Bond	Functional group	Intensity
3358.07	O–H stretch, H–bonded	Phenols	Medium
2966.52	C–H stretch	Alkenes	High
1604.77	N–H bend	1° amines	Medium

From IR graph, it is observed that in highlighted area, the major peak with frequency range of 3358.07^{-1} indicates presences of eugenol and higher peak area for alkene indicates presence of caryophyllene.

CLOVE OIL FTIR

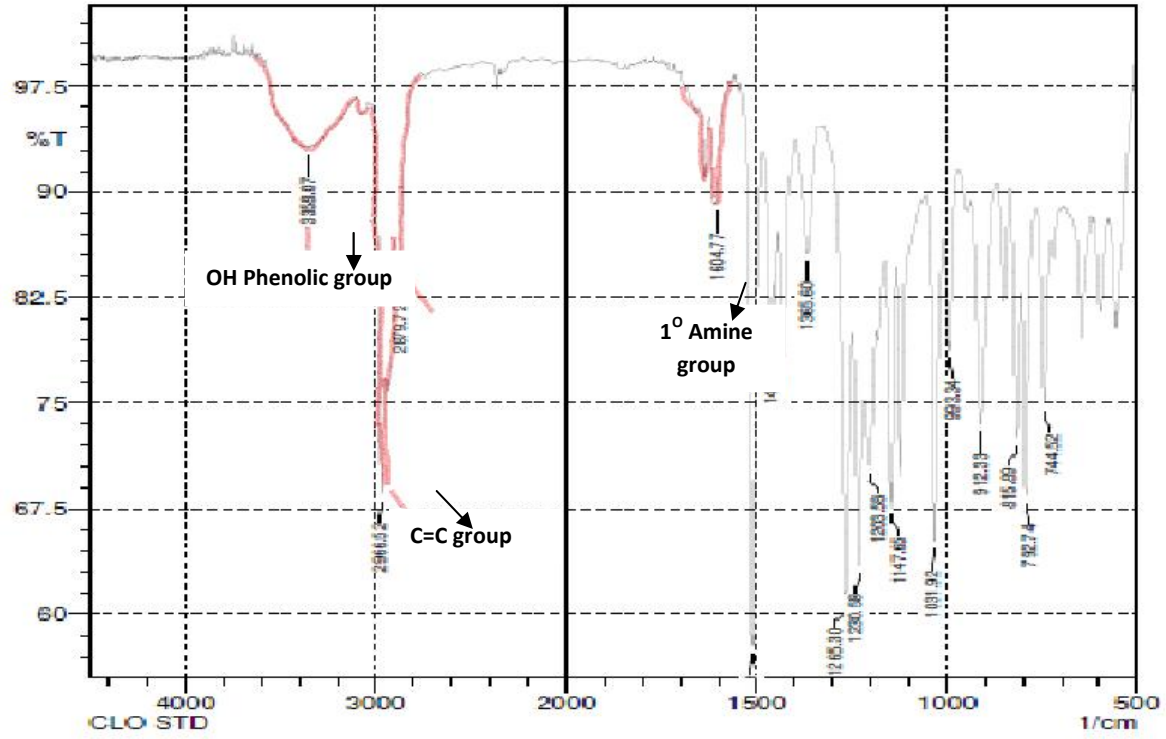


Figure1: IR graph of clove oil.

b. EUCALYPTUS OIL FTIR

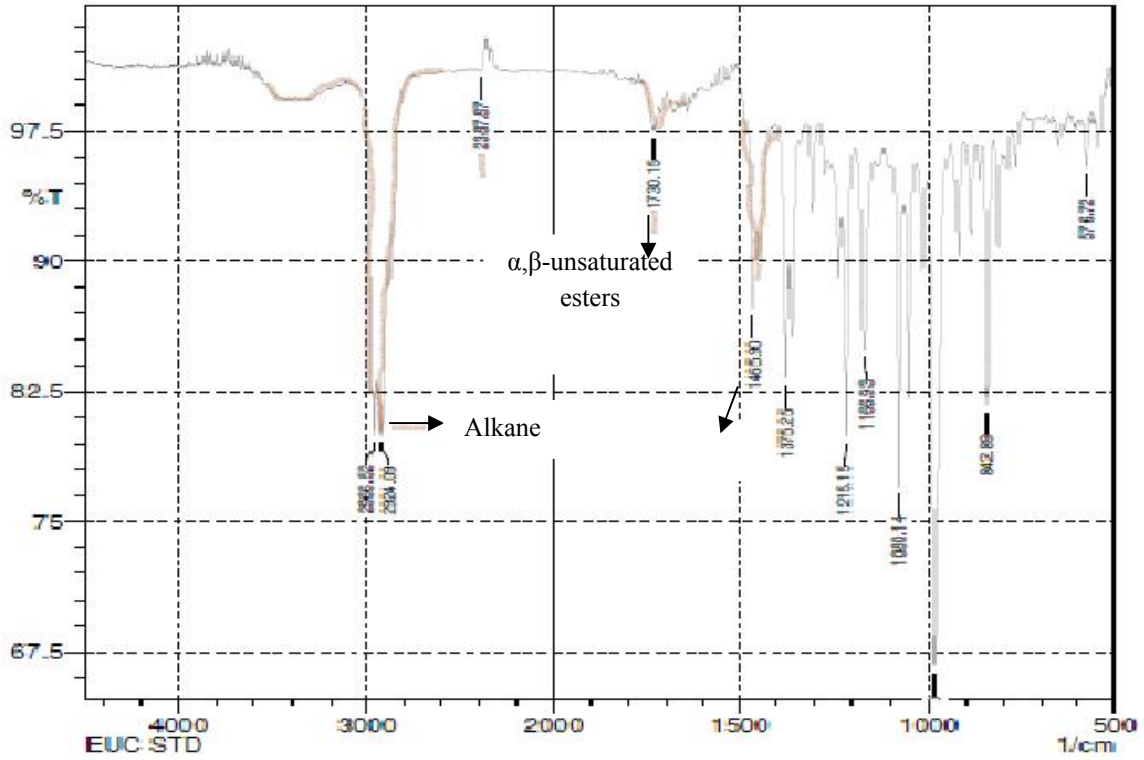


Figure2: IR graph of Eucalyptus oil.

Table6: FTIR Frequency range obtained for Eucalyptus oil.

Frequency cm^{-1}	Bond	Functional group	Intensity
2966.52	C–H stretch	Alkanes	High
1730.15	C=O stretch	α,β-unsaturated esters	Low
1465.9	C–H bend	Alkanes	Medium

From graph, it is observed that, the peak intensity for C=O stretch bond was low as compare to other two peak intensity and this peak was related with oxygenated component of eucalyptus oil such as cineole, limonene etc. The first peak with high intensity related with maximum percentage of hydrocarbon in an oil.

c. NEEM OIL FTIR

The FT-IR spectra in the mid-infrared region have been used to identify functional groups and the bands corresponding to various stretching and bending vibrations in the samples of oil. The position of carbonyl group in FT-IR is sensitive to substituent effects and to the structure of the molecule. The methoxy ester carbonyl group was appeared at 1743.65 cm^{-1}. The band appeared at 3006 cm^{-1} showed the hydrocarbon stretch of aromatic group. The C-H wagging vibration showed at 1171 cm^{-1} and C-H rocking vibration observed at 721.38cm^{-1}. (Table 7).

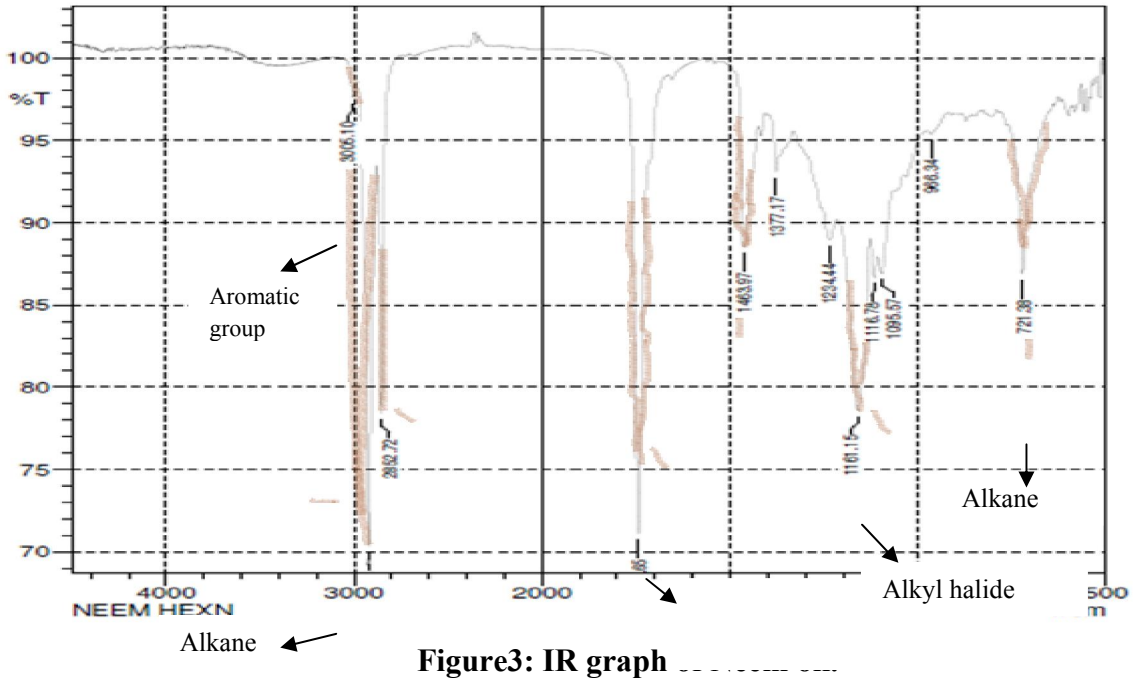


Figure3: IR graph

Table7: FTIR Frequency range obtained for Neem oil.

Frequency cm^{-1}	Bond	Functional group	Intensity
3006.1	C-H Stretch	Aromatic	High
2922.16	C-H Stretch	Alkanes	Medium
1743.65	C=O Stretch	Esters, saturated aliphatic	Medium
1463.97	C-H Bend	Alkanes	Low
1161.15	C-H Wag	(CH_2-X) alkyl halides	Medium
721.38	C-H Rock	Alkanes	High

d. Camphorated coconut oil

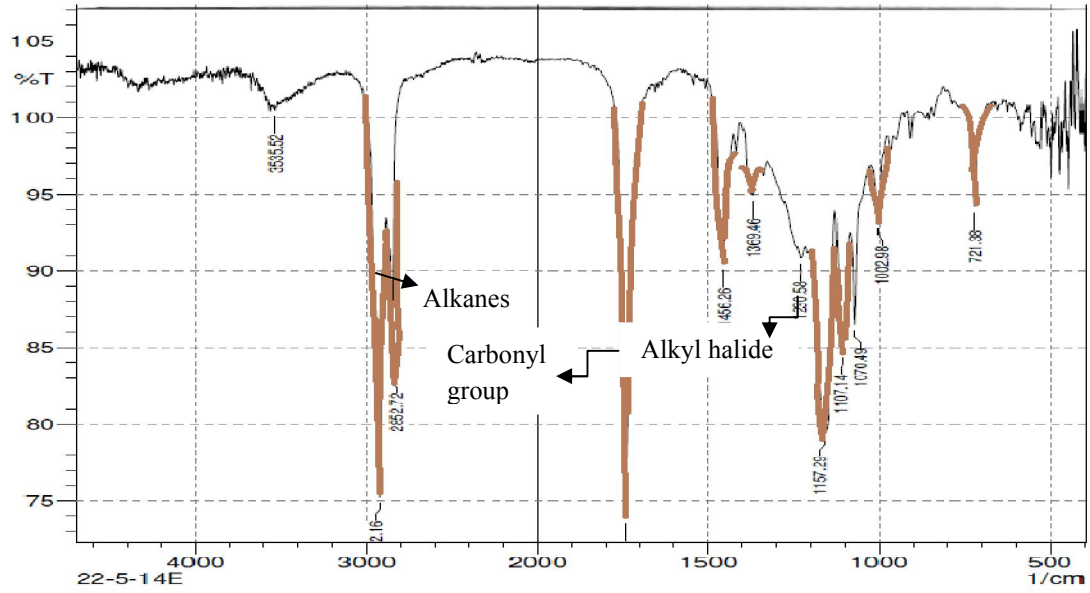


Figure4: IR graph of camphorated coconut oil.

Table8: FTIR Frequency range obtained for Camphorated coconut oil.

Frequency cm^{-1}	Bond	Functional group	Intensity
2922.16	C–H stretch	Alkanes	Strong
1743.63	C=O stretch	Carbonyls	Strong
1456.26	C–H bend	Alkanes	Medium
1369.96	C–H rock	Alkanes	Medium
1157.29	C–H wag	($–CH_2X$) Alkyl halides	Medium
1107.14	C–N stretch	Aliphatic amines	Low
721.28	C–H rock	Alkanes	Low

IR spectrum for camphorated coconut oil shown in Figure8, exhibits a strong band appearing at 2922.16 cm^{-1} assigned to the stretching vibration of hydrocarbon group as due to stretching of CH_3 group, 1743.63 cm^{-1}due to the C=O functional group as carbonyl. In the region 1369 cm^{-1}due to C-H in rocking vibration, 1157 cm^{-1}due to alkyl halide group region, a low band 1107 cm^{-1} due to C-N stretching in linear amine produce region and then at 721 cm^{-1} in plane due to the C-H rocking of hydrocarbaon.

6.3.2 GAS CHROMATOGRAPHY OF ESSENTIAL OIL

a. Clove oil

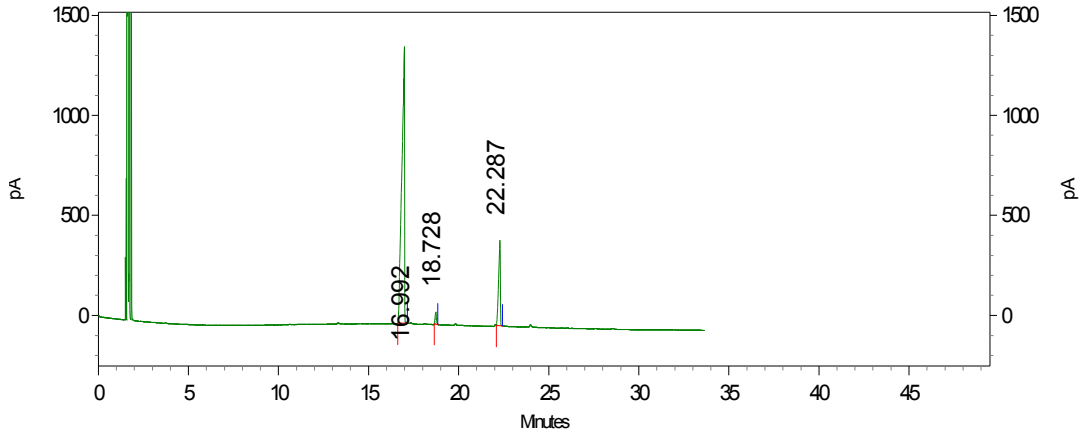


Figure5: GC graph of clove oil.

Clove bud oil were analyzed by GC and 3 main constituents were identified and quantified. The major constituents of bud oils were eugenol (83.60%) and β–caryophyllene (14.84%) and minimum amount of eugenyl acetate (1.56%).

b. Eucalyptus oil

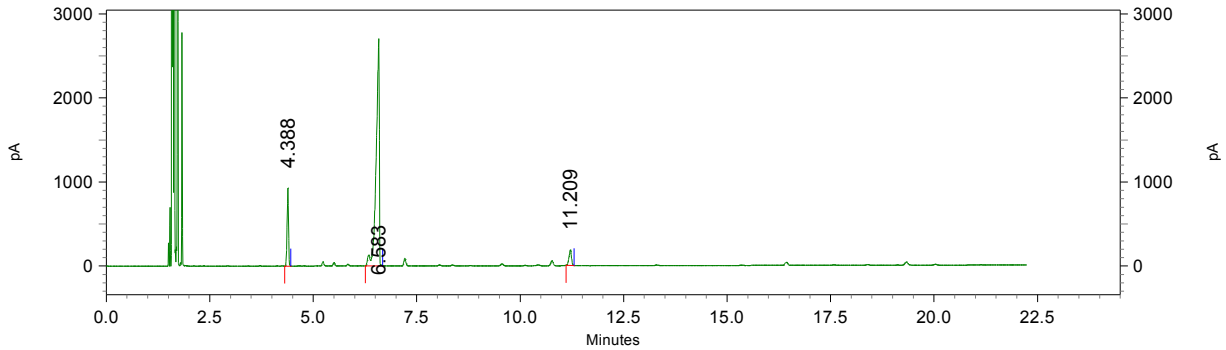


Figure6: GC graph of eucalyptus oil.

Table.9: GC retention time of Eucalyptus oil.

Retention Time	Area	Area %
4.388	17666617	13.48
6.583	107378614	81.93
11.209	6020616	4.59

The major peak obtained at 6.5min indicates presence of cineole.

6.4 ACTIVTY OF OIL

6.4.1 Oils activity (Bioassay)

The contact insecticidal activities of different oils at a dose of 0.0625, 0.125, 0.25 and 0.5 mg/cm^2 against P. humanus capitis were studied and compared clove oil, eucalyptus oil, neem oil and camphorated coconut oil result (Table 10). Significant differences were observed in the contact toxicity to head lice. In particular, clove oil was 2.0-fold more toxic than eucalyptus. No mortality was observed for solvent-treated lice over the observational interval of the contact bioassay. Clove oil was 50-fold more active in the fumigant assay than eucalyptus oil.

6.4.2 Pediculicidal activity of Oil of different concentration

In the individual oil activity against head-lice, clove oil have highest pediculicidal activity in mean death time of 24.49 min as compare to other oil like eucalyptus, neem and camphorated coconut oil as 97.81, 111.62 and 149.40 respectively.

The clove oil has higher activity against p.humans captis because chemical component of clove as eugenol have higher synergetic activity.

The overall result of oils with different concentration as shown below:

Table10: In vitro Pediculicidal activity of Individual oil.

S. No	Ingredients	Different concentration(mg)				
		0.0625	0.125	0.25	0.50	
		Death time(min)				Mean Death Time
1.	Clove oil	19.20	31.28	21.31	26.19	**24.49**
2.	Eucalyptus oil	240.37	120.77	12.06	18.05	97.81
3.	Neem oil	300.54	8.41	120.43	17.13	111.62
4.	Camphor in coconut oil	240.18	180.55	56.49	120.40	149.40
	Total	**800.29**	**340.01**	**210.29**	**181.77**	**383.32**

Form study, clove oil has maximum activity response within minimum concentration of oil as at 0.25mg; the activity within 21.31min and also mean death time of lice was 24.49min for clove oil as compare to other oil. For eucalyptus oil, with decrease in concentration of oil there was increase in activity of oil against head lice and it shows higher activity at concentration of 0.25mg as 12.06mins. but overall mean death time was less as compared to clove oil. For neem oil it is observed that, it has higher resistance activity as compared to their pediculicidal activity and it shows maximum activity within 8.41mins at 0.125mg concentration of oil.

Camporated coconut oil was consider as almost last because it takes maximum time and shows repellent as well as pediculicidal activity. It takes 54.49mins for 0.25mg concentration of oil and in series of concentration; this is best one concentration of camphorated coconut oil for their activity.

7. CONCLUSION

High quality of essential oil is obtained by using hydro distillation method and non-edible or neem oil obtained through solvent extraction process under laboratory conditions. The main component of clove and eucalyptus essential oil is found to be more active and have lethal activity against head lice. It was observed that, at different milligram doses or concentration of essential oil shows resistance as well as death rate activity against head lice. When considering neem oil and camphorated coconut oil, it is found that it shows higher resistance activity than pediculicidal activity. Maximum overall acceptability was observed at lower concentration of clove oil at 0.0625mg for lice as compared to eucalyptus oil. The clove oil activity mainly due to active phenolic component and that related with synergetic activity in it. From our research study, it is observed that at minimum concentration of clove oil, it show higher toxicity to head lice as compared to three other oil.

8. REFERENCES

1. Gratz N G, Human lice: their prevalence, control and resistance to insecticides-a review. World Health Organization, Geneva, Switzerland, WHO CTD WHOPES, 97(8): 1985-1997.
2. Rozendaal J A, Methods for use by individuals and communities. World Health Organization, Geneva, Switzerland. 1997.
3. Dolianitis C and Sinclair R Optimal treatment of head lice: is a no-nit policy justified? Clin. Dermatol, 2002; 20: 94 -96.
4. Burgess I F, Annu Rev Entomol, 2004: 49:457.
5. Isman M B, Pesticides based on plant essential oils. Pestic. Outlook, 1999; 68-72.
6. Young-Cheol Yang, Hoi-Seon Lee, J M Clark, Young-Joon Ahn Insecticidal Activity of Plant Essential Oils Against Pediculus humanus capitis (Anoplura: Pediculidae). J. Med. Entomol, 2004; 41(4): 699-704.
7. Croteau R, Kutchan T M, Lewis N G, Natural products (secondary metabolites). In: Buchanan, B., Gruissem, W., Jones, R. (Eds.), Biochemistry and Molecular Biology of Plants. American Society of Plant Physiologists, 2000.
8. Betts T J, Chemical characterisation of the different types of volatile oil constituents by various solute retention ratios with the use of conventional and novel commercial gas chromatographic stationary phases. J. Chromatogr, 2001; A 936: 33–46.
9. Bowles E J, Chemistry of Aromatherapeutic Oils. Allen & Unwin, ISBN 174114051X, 2003.
10. Pichersky E, Noel J P, Dudareva N, 2006. Biosynthesis of plant volatiles: nature's diversity and ingenuity. Science 311, 808–811.
11. http://hairgrowthoil.net/
12. Flower and Vegetable Oils, R.E.D. Facts, EPA
13. Pharmacopoeia of India, Volume-II, 1985, A-124, A-137.
14. Sofowora A, Medicinal plants and Traditional Medicine in Africa. Spectrum Books, Ibadan, 1993; 150.
15. Trease G E, and Evans W C, Pharmacognosy. 13th edn. Bailliere Tindall, London, 1989; 176-180.
16. Pelczar M J, Chan ECS and Krieg NR. Control of microorganisms, the control of microorganisms by physical agents. In: Microbiology. McGraw-Hill International, New York, 1998; 469-509.

17. Dorman HJD, Deans SG. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. J. App. Microbiol, 2000; 88: 308-316.
18. Polterait O, Antioxidants and free-radical scavengers of Natural Origin. Current Org. Chem, 1997; 1: 415-440.