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A PEDICULICIDAL ACTIVITY OF CLOVE OIL

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ABSTRACT: The humans head lice are a nuisance for millions of people worldwide with high prevalence in children. Head lice have been treated by methods that include the physical removal of lice, various domestic treatments and conventional insecticides. None of these methods render complete protection and there is clear evidence for the evolution of resistance and cross-resistance to conventional insecticides. 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. 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 that have high medicinal value and therefore use against head lice as Chemical constituents of essential oils present a wide range of biological activities. The aim of this work was to evaluate insecticidal activity of essential oil specially clove oil and eucalyptus oil and compare the relative toxicity of essential oil. In the present study, it is observed that from literature survey it is given that eucalyptus has higher toxicity than clove but practically clove oil have higher toxicity as compare to eucalyptus oil to head lice was found. This essential oil was obtained by distillation process and components were identified by FTIR and GC.

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.

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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 and 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 essential oils and their chemical components 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.

Plant essential oils have been suggested as an alternative source of materials for insect control because they constitute a rich source of bioactive chemicals and are commonly used as fragrances and flavoring agents for foods and beverages [5]. Because of this, much effort has been focused on plant essential oils or phytochemicals as potential sources of commercial head lice control agents specially clove and eucalyptus oil as from literature survey it is observed that eucalyptus have higher relative toxicity as compared to clove [6] therefore this research mainly focus on higher toxicity with minimum concentration of essential oil and comparison of both essential oil for head lice.

TABLE1: RELATIVE TOXICITY OF ESSENTIAL OIL[6] (LITERATURE SURVEY)

Essential oil	Relative toxicity
Clove bud	1.2
Eucalyptus	5.5

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].

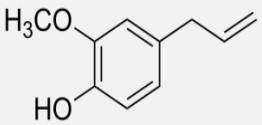
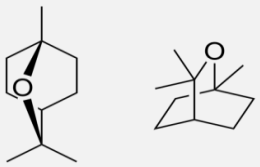
Clove oil:

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 support for hair growth stimulation [11].

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.

TABLE 2: ESSENTIAL OIL AND THEIR MAIN CONSTITUENTS

Sr.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 

MATERIALS AND METHODS:

Insects:

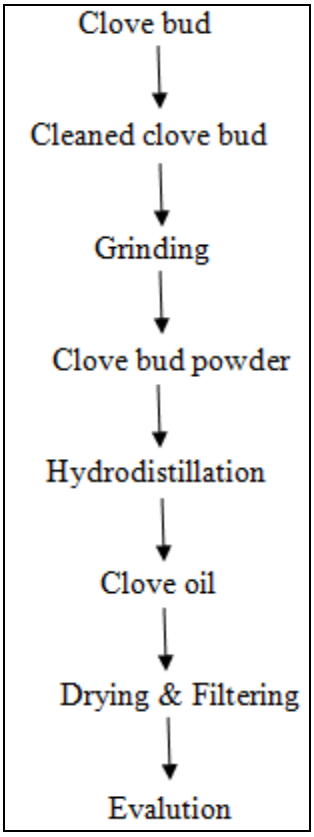
Head lice were collected from children of 6-13 years old, using a fine toothed comb. Lice were obtained from slum area, Mumbai, where a topical method indicated high resistance levels to permethrin. After collection, head lice were freshly collected and placed in an environmental chamber at 18 ± 0.5ºC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

Plant material:

The raw material clove and eucalyptus consist of buds and leaves. The clove bud purchased from the local market and eucalyptus leaves obtained from campus of ICT, Mumbai. The material was naturally dried in shadow and stored in controlled laboratory conditions.

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. 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%.



ANALYSIS:

Refractive index [13]:

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.

Specific gravity or weight per milliliter [13]:

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.

Moisture:

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

Phytochemical screening

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

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.

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.

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.

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.

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

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.

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.

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.

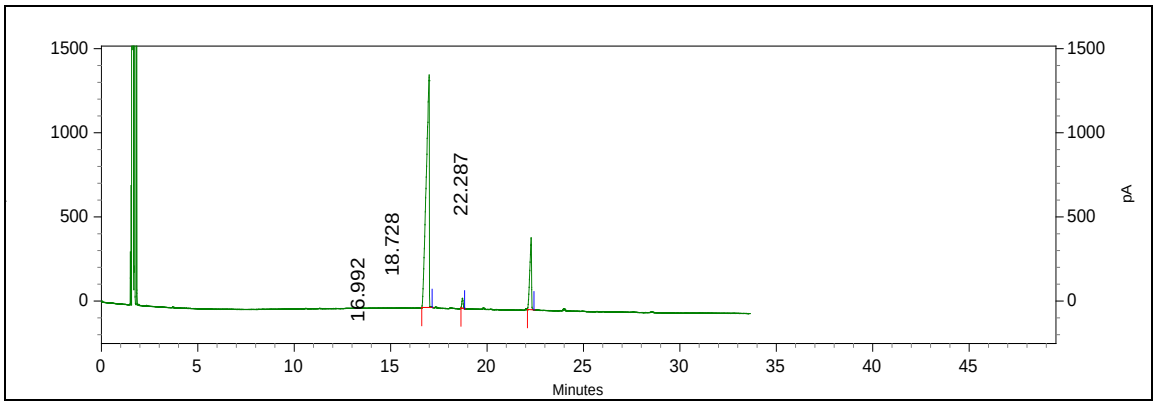


FIGURE 1: GC GRAPH OF CLOVE OIL

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%).

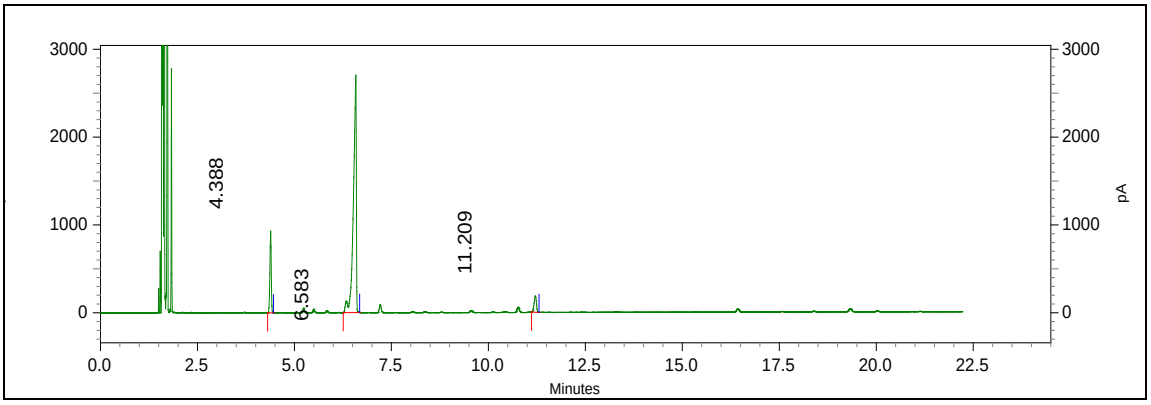


FIGURE 2: GC GRAPH OF EUCALYPTUS OIL

TABLE 3: 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.

Fourier Transform Infrared Spectroscopy (FTIR) of Essential oil: It related with physical and chemical methods of analysis for the 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.

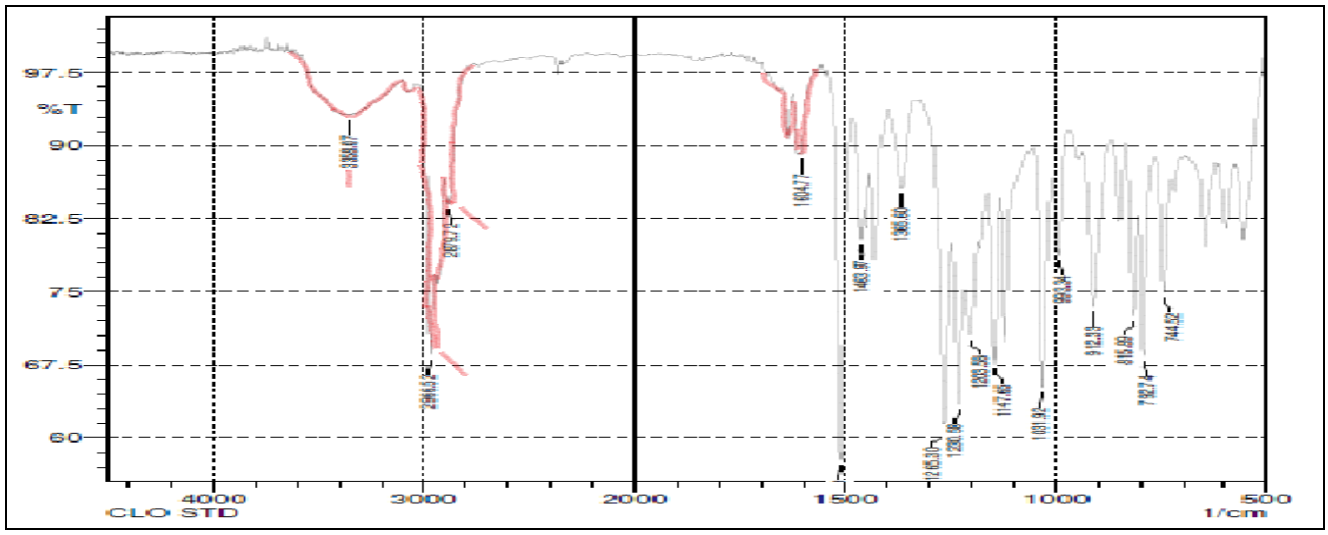


FIGURE 3: IR GRAPH OF CLOVE OIL

TABLE 4: FTIR FREQUENCY RANGE OBTAINED FOR CLOVE OIL

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

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.

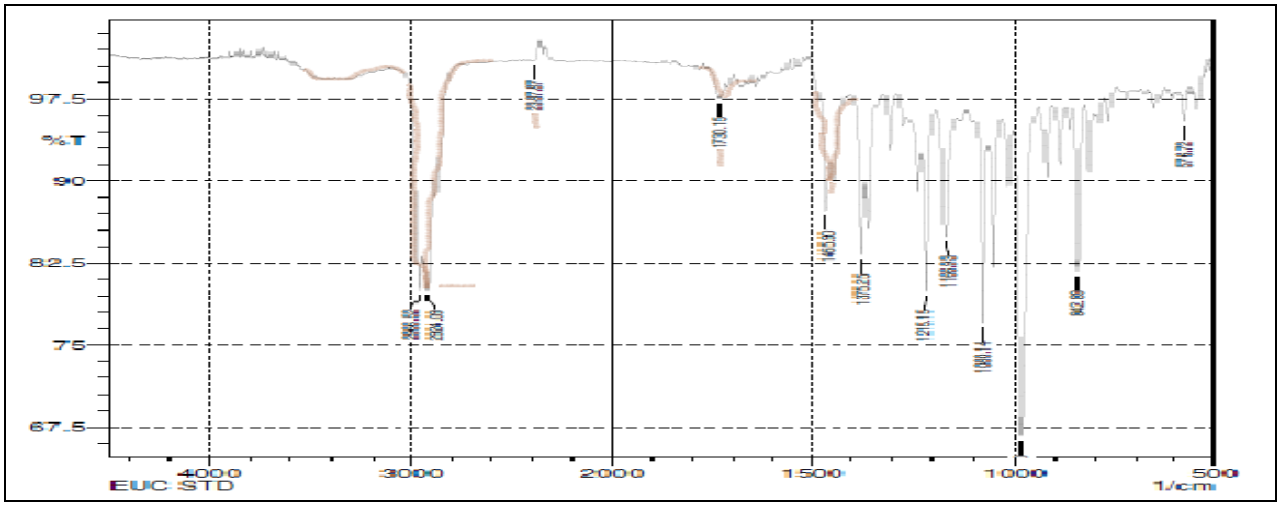


FIGURE4: IR GRAPH OF EUCALYPTUS OIL

TABLE 5: FTIR FREQUENCY RANGE OBTAINED FOR EUCALYPTUS OIL

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

Chemicals and Essential Oils:

Essential oils were obtained through hydro-distillation process. All other chemicals were of reagent grade and purchased from market. Head Lice a colony of *P. humanus* capitis was freshly collected from infested children from slum area. Head lice were reared in petri dishes (4.5 cm in diameter) with 0.01- and 1.0-mm mesh screens attached over the central holes (4 cm in diameter) on the lid and bottom sides respectively and containing a few strands of human hair. Freshly feed head lice collected and use for experiment.

Bioassay:

A filter paper contact bioassay was used to evaluate the toxicity of the essential oils and insecticides to *P. humanus* capitis. In a preliminary experiment with clove bud, eucalyptus 0.0625 mg/cm^2 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 essential 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.

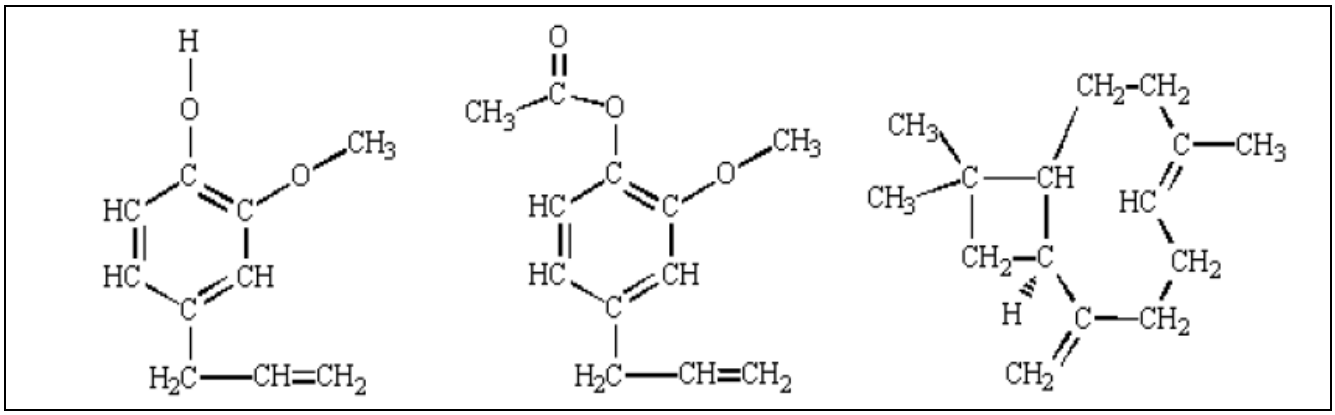
RESULTS AND DISCUSSION:

The oil obtained in hydro-distillation process was analyzed by means of checking their properties as follow:

TABLE 6: PROPERTIES OF ESSENTIAL OIL

Properties ↓ /oils →	Clove	Eucalyptus
State	Liquid	Liquid
Color	Colorless-light yellow	Colorless
Odour	spicy	Aromatic
R.I	1.5272	1.4564
Sp. Gravity	1.045	0.920
Solubility	Methanol and Diethyl ether	Slightly in methanol
Moisture content of raw	16.12%	12.73%
% oil	13.86%(25)	3.48%(20)

The raw material for oil yield have moisture content 16.12% with that oil yield 13.86% for 25gm of raw clove bud in hydro distillation. The oil obtained was colorless to yellow in colour with a specific gravity of 1.045 and a refractive index value of 1.5272. The Oil found to have potent repellent as well as pediculosidal activity against *pediculus humanus* capitis organisms tested. They are soluble in different oraganic solvent as in methanol, diethyl ether, ethyl acetate etc.



EUGENOL **EUGENOL ACETATE** **CARYOPHYLLENE**

FIGURE5: CHEMICAL STRUCTURES OF THE COMPONENTS OF CLOVE OIL (EUGENOL, EUGENOL ACETATE AND CARYOPHYLLENE).

From GC of clove oil it is observed that, the peak obtained at 16.99 and 22 min were eugenol and caryophyllene high area peak as Eugenol is a phenolic compound. Phenols are known to have antiseptic properties [16], which is consistent consistent with the antimicrobial data obtained for these compounds. Caryophyllene **(Figure 5)** has also been shown to possess pediculosidal properties, though not as potent as eugenol [17].

Phytochemical screening of plant materials:

The phytochemical screening of the essential oil studied showed the presence of reducing sugar and terpenoids **(Table 7)**. Clove oil and eucalyptus 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 are 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.

TABLE 7: PHYTOCHEMICAL RESULTS

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

Essential oil activity (Bioassay):

The contact insecticidal activities of essential 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 and eucalyptus oil result **(Table 8)**. Significant differences were observed in the contact toxicity to head lice. On the basis of relative toxicity values of different essential oil, two essential oil were selected. 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.

TABLE 8: CLOVE AND EUCALYPTUS OIL BIOASSAY

Quantityof oil(mg)	Time(min)	
	Clove oil Oil	Eucalyptus Oil
0.0625	19.2	240.37
0.125	31.28	157.4
0.25	21.31	12.06
0.5	26.19	18.05

From study, it is observed that clove oil has higher resistance and toxicity against head lice as compared to eucalyptus oil from **Tables 8** at different oil concentration.

CONCLUSION: High quality of essential oil is obtained by using hydro distillation method 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, head lice show there resistance as well as death rate 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 active its phenolic component and that related with synergetic activity in it. From literature survey, it is given that eucalyptus oil has higher relative toxicity as compared to clove but from our research study, it is observed that at minimum concentration of clove oil, it show higher toxicity to head lice.

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