Phytochemical Analysis of Various Essential Oils of Plant Extracts and Its Effect on Mosquito Repellent Activity

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Abstract:

Mosquito causes severe human health diseases which may cause death of humans. It is responsible in causing sever diseases such as malaria, dengue, elephantitis etc. Thus, to overcome this problem many chemical repellents are being used. Longer time use of these chemical based repellent causes health issues that would be very harmful to humans. Thus, in this current investigation we are formulating herbal based mosquito repellent in order to avoid skin related problems, neurological disorders, and other health issues. The plants are the reservoirs of naturally occurring chemical compounds and structurally diverse bioactive molecules. Plants are recognized for their broad spectrum of pharmacological activities. The biological activities as secondary metabolites and phytochemicals derived from different plant parts as leaves, flowers, seeds, bark, root, and pulp. Current investigation suggests the beneficial use of various phytochemicals, essential oils and plant extracts that are used to kill mosquito. These phytochemicals such as alkaloid , flavonoid , saponoid , tannins , cardiac glycoside , glycoside , terpenoids , phenols , coumarins , phytosterol . These phytochemicals showed beneficial effects to overcome the use of chemical repellents. Our investigation showed alkaloid, flavonoid, saponoid phytochemicals as the best effective measure for killing of mosquito. Nature plant extracts and essential oils such as neem oil, camphor, coconut oil, orange peel, lemon grass oil, peeled oil etc. are used to formulate herbal mosquito repellent. Neem oil and lemon grass oil showed the best effective against mosquito. For further testing cage test was employed for Larvicidal activity. As a result, it showed 99% activity against mosquito that were gradually killed by the beneficial effects of essential oils and prominent plant extracts used in cage test.

Keywords: Phytochemicals, Essential Oils, Plants Extract, Cage Test Activity.

Introduction:

Natural products, such as plants extract, open a new horizon for the discovery of new antimicrobial agents. The use of traditional medicine and medicinal plants in most

developing countries, as a normative basis for the maintenance of good health, has been widely observed and about 80% of the world's population relies on herbal medicines. Plants contain a wide range of chemical compounds that can be used to treat chronic as well as mosquito based disease. Microbial resistance from the plant extract gave us new scope of study to do formulation of herbal based plant products by using various plant extract and essential oils. They found literally thousands of phytochemicals proved beneficial and have biological activity such as anticancer, antimicrobial, antioxidant, ant diarrheal, analgesic and wound healing activity were reported. The medicinal power of traditional plants species lies in phytochemical components that cause definite pharmacological action on the human body (Naseem 2014). Based on their metabolism activity in the plant, phytochemicals components are generally can be mainly divided into two groups, which are primary which has mainly sugars, amino acids, chlorophyll and proteins, and secondary constituents while secondary constituents consist of alkaloids, flavonoid, saponins, tannins, phenolic compounds and many more (Krishnaiah 2007).

Plants are recognized in the pharmaceutical industry due to their broad spectrum of structural diversity and their wide range of pharmacological activities. The biological active compounds that are present in plants referred as phytochemicals. These phytochemicals derived from different parts of plants such as leaves, barks, seed, seed coat, flowers, roots and pulps and thereby used as sources of direct medicinal agents. Phytochemistry describes the large number of secondary metabolic compounds present in the plants. The plants are the reservoirs of naturally occurring chemical compounds and of structurally diverse bioactive molecules. (Krishnaiah 2007).

Synthetic mosquito repellents are causing damage to ecosystem and harmful effect for human beings also. People now a days are migrating towards herbal mosquito repellent in order to avoid their skin damage and avoid various neurological disease herbal mosquito repellent are available in market in larger quantity but there prices are costly. so in order to overcome these drawback regarding cost of herbal mosquito repellent we have made an herbal mosquito repellent which can kill larvae and mosquito due to presence of certain secondary metabolites in them namely alkaloid, flavonoid, saponoid, tannins, cardiac glycoside, glycoside, terpenoids, phenols, coumarins, phytosterol. Presence of certain secondary metabolite has been done by doing phytochemical analysis of various plant extract

Many studies reported plants as safe and broadly effective alternatives with fewer adverse effects Mosquito repellent product and chemical based mosquito repellent devices available in the market of reported to have harmful effects on human being; the present study is to develop effective plant based mosquito repellent products. The essential oils have toxic effect for mosquitoes and other insects and high repellency. It can inhale, ingested or skin observed by insects. Herbal extracts are used as a natural alternative to DEET (N, N diethyl Meta toluamide). Herbal mosquito repellent (plant extract, essential oils) formulation may be an effective, inexpensive, and to decrease the mosquito borne diseases like as malaria, dengue etc. (Krishnaiah 2007).

Key words: Phytochemical analysis of Plant extract, Neem Leaves, Orange Peels, Essential oils, Mosquito Larvae, Formulation of Herbal Mosquito Repellent.

Materials and methods:

1. Collection of plants extracts oils from various ayurvedic shops of Rajkot.
2. Phytochemical Analysis of plant extracts.
3. Mosquito Larvicidal Activity and repellent activity by Cage test.

1. Collection of plants extracts oils from various ayurvedic shops of Rajkot:

Raw materials were collected from various plant parts – Orange (*Citrus sinensis*) peels, Neem (*Azadirachta indica*) leaves, Coconut oil (Cocus nucifera), Camphor($C_{10}H_{16}O$), Sandalwood (Santalum album), Garden Rose (Rosa chinensis), Jasmine(Jasminum multifloram), Peeled lemon(Citrus limon) and Lemongrass(Cymbopogon citratus) were collected from local market of Kalawad Road and Atmiya University Campus at Rajkot(Ranasinghe *et al*., 2016).

2. Phytochemical Analysis of plant extracts:

- TEST OF ALKALOIDS: Add 2.0 ml of test solution and add few drops of Mayer's Wagner reagent presence of precipitation shows alkaloids. (Ibrahim,et.al 2020)
- TEST OF COUMARINS : Add 2.0 ml naoh + 2.0 ml test solution development of blue or greenish yellow color (Ibrahim,et.al 2020)

- TEST OF FLAVOINOIDS : Add 1.0 drop of lead acetate + 1.0ml test solution formation of yellow color(Ibrahim,et.al 2020)
- TEST OF TANNINS : Add 2.0 ml of test solution + 2.0 drops of dilute ferric chloride solution color changes from green to blue green or blue black (Ibrahim,et.al 2020)
- TEST OF SAPONOIDS: Add 2.0 ml test solution+2.0 ml distilled water shake vigorously for 15 sec persistent frothing (Ibrahim,et.al 2020)
- TEST OF CARBOHYDRATE : 0.1gm test solution was shaken vigorously with 5.0 ml distilled water and add few drops of molisch reagent followed by vigorous shaking concentrated sulphuric acid 1ml was added carefully to form a layer below the aqueous solution a brown ring at interface indicates the presence of carbohydrate. (Ibrahim,et.al 2020)
- TEST OF REDUCING SUGAR: 0.1gm test solution was shaken vigorously with 5.0ml distilled water and filtered add equal amount of Fehling's solution A and Fehling's solution B. brick red precipitates indicates the presence of reducing sugars. (Ibrahim,et.al 2020)
- TEST OF PHYTOSTEROL: Add 2.0 ml of test solution with 2.0 ml of concentrated sulphuric acid was shaken for few minutes and mixed well development of red or brown color indicates presence of sterols. (Ibrahim,et.al 2020)
- TEST OF AMINO ACID : Add 1.0 ml of test solution in test tube add few drops of ninhydrin reagent appearance of purple color indicates presence of amino acid (Ibrahim,et.al 2020)

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- TEST OF ANTHRAQUINONES: Add 1.0ml of test solution with 0.5ml concentrated sulphuric acid then leave it for 5 mins and add ammonia formation of rose pink color indicates presence of anthraquinones (Junaid R Shaikh, et.al 2019).

- TEST OF LEAD ACETATE: Add 2.0 ml of the test solution add 2.0 ml of 20% Naoh and boil for a minute Cool it and add a drop of lead acetate solution formation of black lead sulfide precipitate. (Junaid R Shaikh, et.al 2019)
- TEST OF CARDIAC GLYCOSIDE: Glycoside is dissolved in a mixture of 1 % ferric sulphate solution in (5%) glacial acetic acid. Add one or two drop of concentrated sulphuric acid. A blue color develops. (Junaid R Shaikh, et.al 2019)
- TEST OF GLYCOSIDES: Add 2.0 ml test solution+4.0 ml glacial acetic acid containing 5% ferric chloride solution which was inlaid with 1ml concentrated sulphuric acid. brown ring at interface (Junaid R Shaikh, et.al 2019)
- TEST OF ANTHOCYANIN : Add 2.0 ml mixture of (1.0 ml) hcl and (1.0ml) ammonia into 1.0 ml test solution in test tube color change from pink red to blue violet (Junaid R Shaikh, et.al 2019)
- TEST OF TERPENOIDS : Add 2.0 ml of chloroform mixed with 2.0 ml test solution add 2.0 ml concentrated sulphuric acid and heat for 120s in water bath at 65 degree Celsius reddish brown color appear (Junaid R Shaikh, et.al 2019)
- TEST OF PHENOLS : Add1.0 ml of test solution with 2 drops of ferric chloride formation of deep blue or black color (Junaid R Shaikh, et.al2019)
- TEST OF RESINS: Add 0.2gm test solution treat it with15ml 96% ethanol. Then spoor it into 20ml distilled water and add 3.0 ml of concentrated hcl heat mixture in water bath for 30 min magenta red color(Junaid R Shaikh, et.al2019)
- TEST OF PROTEINS: Add 0.1gm test solution in 5ml distilled water this was left to stand for hours and then filtered. 2.0 ml portion of filtrate was added to 0.1ml Million’s reagent. Shaken and kept for observation yellow color(Junaid R Shaikh, et.al2019)
- TEST OF QUINONENS: A small amount of extract was treated with concentrated HCL and observed for the formation of yellow precipitate(Junaid R Shaikh, et.al2019)

3. Mosquito Larvicidal Activity and Mosquito repellent activity by Cage test.

Larvae cultures were collect from botanical garden of Atmiya University Campus Rajkot, Gujarat. The larvae cultures have been maintained in glass bottle containing tap water. All the experiments were carried out at 30°-35°C. Fish food was feed to larvae cultures for the fittest of survival (Patil *et al.,* 2010)

- ✓ For the study of Larvicidal activity total volume of 20.0ml essential oil extract with distilled water has been prepared. 2.0ml essential was used for oil suspension it makeup with 18.0ml of distilled water. After completion of prepared oil suspension it was poured into the plastic box for experiment conduction. Then added 30 mosquito larvae in plastic box of oil suspension. In this investigation each and every raw materials were used separately at various ratio. Then observed mortality of larvae at every 5 minutes of class interval.

- ✓ The test repellant solution was checked for its killing efficiency employing LC50 (Patil *et al.,* 2010) against the mosquitoes. The plastic cage size is 33.4× 25.8 ×16.4 cm. The cage needs to be filled with 30 mosquitoes and only were supplied with fish food and check the time of LC50 value. Observe the effect of the repellent on the mosquitoes and count the number of dead mosquitoes at different time duration

Observations and Results:

Phytochemical of Plant extracts and Essential Oils:

Phytochemical analysis of plant extract is necessary for qualitative and quantitative estimation of secondary metabolites present in plant. To kill mosquitoes naturally mainly phytochemicals such as alkaloid, flavonoid, saponoid, cardiac glycoside, glycoside, terpenoids, tannins, phytosterol, coumarins and phenols are mainly responsible. Main phytochemical that kills mosquito as we well as larvae are alkaloid, flavonoid, saponoid. These agents are useful to develop effective plant-based mosquito-repellent products. For the extraction of Neem leaves and Orange peel, essential oil, and various plant samples were used. The herbal mosquito repellent shows Larvicidal activity against mosquito larvae. After 80 minutes of the application of repellent, 95% of mosquito larvae were killed by the effect of essential oils. Further Cage test was performed for the killing of mosquitoes; the result showed that 70% of mosquitoes were to obtained dead in the cage after 30 minutes of incubation. Hence,

we can conclude that a combination of plant extract and essential oils is used as a source of mosquito repellent. It can also be used as a biodegradable, eco-friendly compound, cost-effective, and has medicinal properties which are beneficial for human health without any side effects.

Table 1: Phytochemical Analysis of Various Plant Extracts

Sr No.	**Phytochemical test**	**Plant extract**			
		Neem oil	**Camphor**	**Orange pills powder**	**Coconut oil**
1.	Test of alkaloids	Positive	Positive	Positive	Positive
2.	Test of coumarins	NA	Positive	NA	NA
3.	Test of flavonoid	NA	Positive	NA	Negative
4.	Test of tannins	Positive	Negative	Positive	Positive
5.	Test of saponoid	Positive	Positive	Positive	Negative
6.	Test of glycoside	Positive	Positive	Positive	Positive
7.	Test of	NA	Negative	NA	NA
8.	Test of terpenoids	NA	Negative	Positive	Negative
9.	Test of amino acid	NA	NA	Positive	
10.	Test of anthraquinones	NA	NA	Negative	NA
11.	Test of phenol	Positive	Negative	NA	NA
12.	Test of proteins	Positive	NA	Negative	NA
13.	Test of resins	NA	NA	NA	NA
14.	Test of carbohydrate	NA	NA	NA	NA

15	Test of phlobatannins	NA	NA	NA	NA
16.	Test of lead acetate	NA	NA	NA	NA
17.	Test of cardiac glycoside	NA	NA	NA	NA
18.	Test of oligomeric proanthocyanidins / betacyanidins	NA	NA	NA	NA
19	Test of polyphenol/casein	NA	NA	NA	NA
20.	Test of reducing sugar	Positive	NA	NA	NA
21.	Test of oils	NA	NA	NA	NA
22.	Test of phytosterol	Positive	NA	NA	NA

Table 2: Phytochemical Analysis of Various Essential Oils of Plants

Sr No.	**Phytochemical test**	**Essential oils**				
		Jasmine oil	**Sandal-wood oil**	**Rose garden**	**Peeled lemon**	**Lemon-grass oil**
1.	Test of alkaloids	Positive	NA	Positive	Positive	Positive
2.	Test of coumarins	NA	NA	Positive	NA	Positive
3.	Test of flavonoids	Positive	Positive	Positive	Positive	NA
4.	Test of tannins	Positive	NA	Negative	Positive	Negative
5.	Test of saponoid	Negative	Positive	Positive’	Positive	Positive
6.	Test of glycoside	NA	NA	Negative	NA	NA

7.	Test of anthocyanin	NA	Negative	Positive	NA	NA
8.	Test of terpenoids	Positive	Positive	Positive	Negative	Negative
9.	Test of amino acid	NA	NA	NA	Positive	Negative
10.	Test of anthraquinones	NA	NA	Positive	NA	Positive
11.	Test of phenol	NA	Positive	Positive	Positive	NA
12.	Test of proteins	Positive	NA	NA	Positive	negative
13.	Test of resins	NA	NA	NA	Positive	Positive
14.	Test of	Positive	NA	NA	NA	Negative
15	Test of phlobatannins	Positive	NA	NA	NA	Positive
16.	Test of lead acetate	NA	NA	NA	Negative	Negative
17.	Test of cardiac glycoside	Positive	NA	Positive	NA	Positive
18.	Test of oligomeric proanthocyanidins / betacyanidins	NA	Negative	Positive	Negative	Positive
19	Test of polyphenol/casein	NA	Negative	NA	NA	Negative
20.	Test of reducing sugar	NA	NA	NA	Positive	Negative
21.	Test of oils	N A	NA	NA	Positive	Negative
22.	Test of phytosterol	NA	NA	NA	Positive	Negative

Observation Table:

1. Phytochemical Analysis of Plants Extract & Essential Oils

	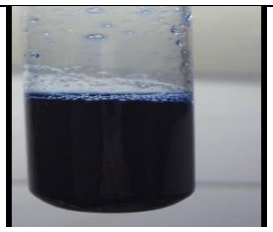	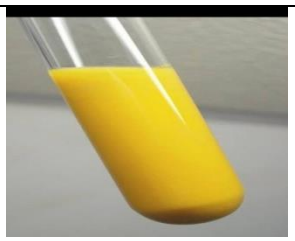		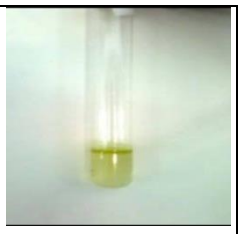
Alkaloid	Coumarins	Flavonoid	Saponoid	Glycoside

Fig 1: Phytochemical analysis of Camphor

	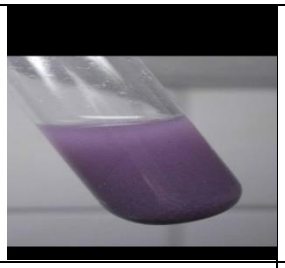	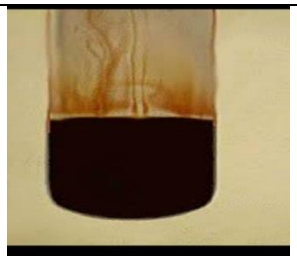		
Alkaloid	Tannins	Saponoid	Terpenoids	Protein

Fig 2: Phytochemical analysis of orange pills powder

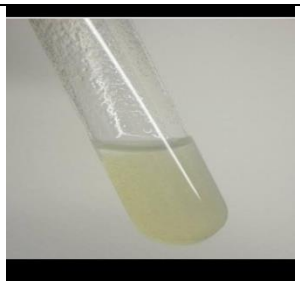			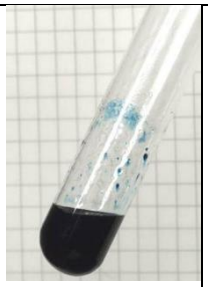	
Alkaloid	Carbohydrate	Glycoside	Protein	Resins

Fig 3: Phytochemical analysis of coconut oil

		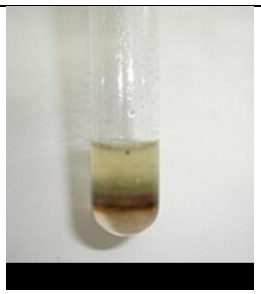		
Phenol	Saponoid	Glycoside	Protein	Sterol

Fig 4: Phytochemical analysis of Neem oil

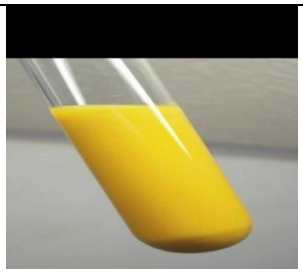		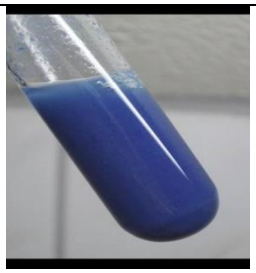		
Saponins	Anthocyanin	Tannins	Cardio glycoside	Reducing sugar

Fig 5: Positive Result Images of Phytochemical analysis for Essential oils

2. Mosquito Larvicidal & Mosquito Repellent Activity Using Herbal Mosquito Repellent:

				
10 mins (Unconscious Mosquito Larve) – Not Significant	15 mins (Least Killed Mosquito Larves) - Significant	20 mins (More Killed Mosquito Larves) - Moderately Significant	25 mins (Least Killed Mosquito Larves) - Highly Significant	30 mins (Least Killed Mosquito Larves) - Very Highly Significant

Fig 6. Larvicidal Activity by employing Herbal Mosquito Repellent (Combination of Prominent Plant Extract and Essential Oil- Lemon-grass)

				
10 mins (Unconscious Mosquito) – Not Significant	15 mins (Least Killed Mosquito) – Significant	20 mins (More Killed Mosquito) - Moderately Significant	25 mins (Least Killed Mosquito) - Highly Significant	30 mins (Least Killed Mosquito) - Very Highly Significant

Fig 7. Mosquito Repellent Activity by employing Herbal Mosquito Repellent (Combination of Prominent Plant Extract and Essential Oil- Lemon-grass)

Table 3: Effect of plant extract on Mosquito Larvae

Experiment	**Number of larvae**	**Number of Killed Mosquito Larvae**						
		00min	**05min**	**10min**	**15min**	**20min**	**25min**	**30min**
Control	30	-	-	-	-	-	-	-
various plant extracts	30	-	+	++	++	++	++++	++++

Where,

Killing Intensity Profile:

0,1 = - (Not Significant), 1-6 = + (Significant), 7-12= ++ (Moderately Significant), 12-20 = +++ (Highly Significant), 20-30 = ++++ (Very Highly Significant)

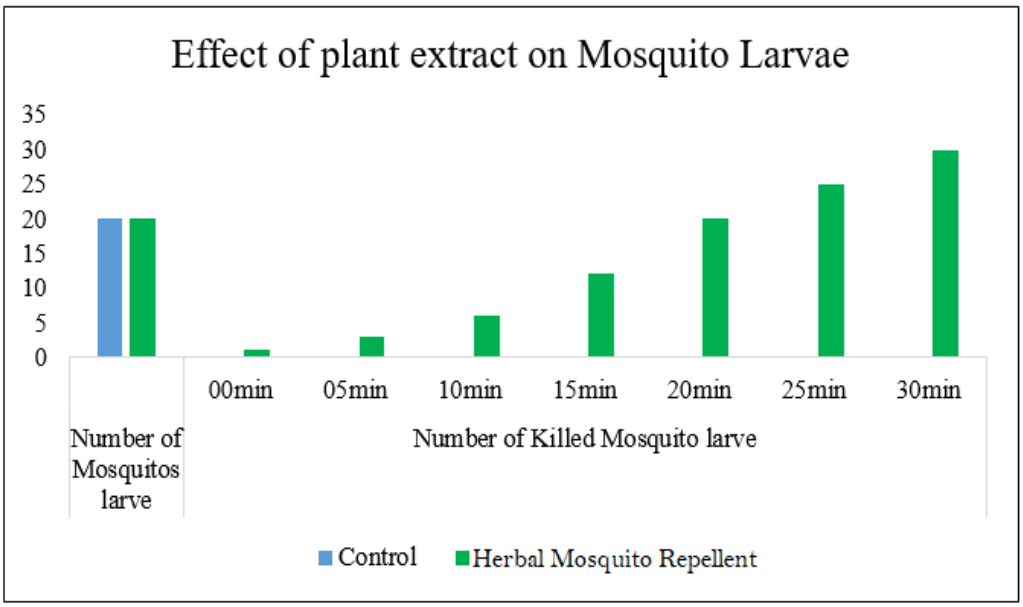

Fig 8: Larvicidal Activity of the plant extract Repellent

Present investigation has carried out by using 30-30 each mosquito larvae's into the cage and observed for 30 mins. Result recorded the highest Larvicidal activity achieved by using **"Herbal mosquito repellent"** has the capacity to kill all 95 to 100% of mosquito larvae between 30 minutes. So, we can consider it is to better mosquito larvae repellent.

Table 4: Effect of various plant extracts on Mosquitoes

Experiment	**Number of larvae**	**Number of Killed Mosquito**						
		00min	**05min**	**10min**	**15min**	**20min**	**25min**	**30min**
Control	20	-	-	-	-	-	-	-
various plant extracts	20	-	+	++	++	++	+++	++++

Where,

Killing Intensity Profile:

0,1 = - (Not Significant), 1-5 = + (Significant), 5-10= ++ (Moderately Significant), 10-15 = +++ (Highly Significant), 15-20 = ++++ (Very Highly Significant)

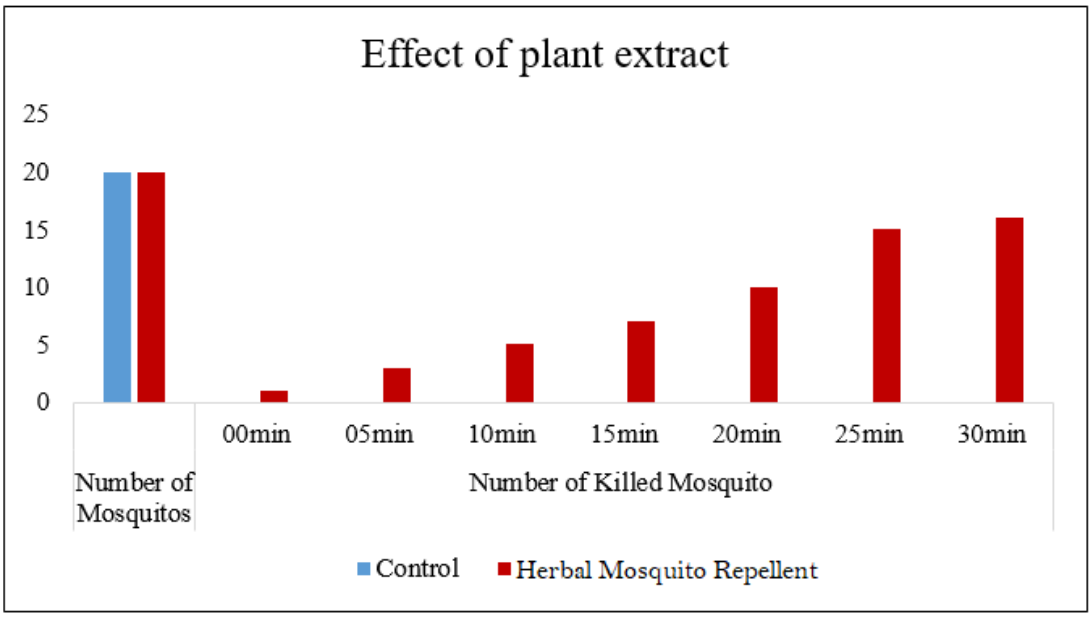

Fig 9: Mosquito Repellent Activity of the plant extract

Present investigation 20-20 each mosquito was kept in the cage for 30 mins observation. Result recorded the highest Mosquito repellent activity at 70% to 80% can mosquitoes killed in between 30 minutes in 30×30 cm cage.

- Hence it has been successfully proven that this "Herbal mosquito repellent" is not only killing larvae but also killing mosquitoes successfully.

Discussion:

Formulation of herbal mosquito repellent from essential oils of medicinal plant extracts done by doing the phytochemical analysis of various plant extract and essential oils. For the test to check the efficacy of essential oils Larvicidal activity for mosquito larvae and after cage test for the how much amount of mosquitoes were kill with time being. (Patil *et al.*, 2010)

The effect of essential oils was toxic for granary insect and also for flying insects. It can be inhaled, ingested or skin absurd by insects As per the recent report herbal mosquito repellent was observe no any harmful effect, skin irritation, rashes, and breathing on human body. It is safe, eco-friendly, low cost, easy to use. In addition, it has maximum repellence against mosquitoes. Herbal mosquito repellents have

increased their quality, safety and efficiency to kill mosquitoes as well as larvae. (Patil *et al.,* 2010)

Many researchers have shown that plant essential oils have better repellent efficacy rather than plant extracts. However, all the essential oils are highly volatile and this contributes to their poor longevity as mosquito repellents. Therefore, many essential oils are not suitable to be used as sole mosquito repellents. This problem is addressed in the current study by preparing herbal mosquito repellent formulations using highly volatile essential oils together with herbal extracts (Patil *et al.,* 2010)

Formulation of herbal based mosquito repellent proved to kill mosquitoes and larvae due to presence of certain plant based secondary metabolites phytochemical in them. Mostly essential oils were having most of the phytochemicals of secondary metabolite responsible for fragrance and plant extracts like Neem oil, coconut oil etc were having phytochemicals of secondary metabolite responsible for producing pungent smell for mosquitoes which cause them inhalation problems .hence mosquitoes were killed by the combination of essential oil and plant extraction in appropriate ratios. (Patil *et al.,* 2010)

Conclusion:

Hence, we conclude that by doing phytochemical analysis of Neem oil, coconut oil, camphor, orange pills powder, sandalwood, jasmine, rose garden, peeled lemon, lemongrass. Mosquitoes can be killed due to presence of different phytochemicals in different plant extracts and essential oil which was isolated from plants.

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