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PHYTOCHEMICAL ANALYSIS AND ANTI-HEAD LICE ACTIVITY OF *AZADIRACHTA INDICA* (A.Juss) AND *AEGLE MARMELOS* (Corr.) (LEAVES)

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ABSTRACT

The aim of study is evaluate the phytochemical analysis and anti-head lice effect of leaves extracts from *Azadirachta indica* (A.Jusss) (Meliaceae) and *Aegle marmelos* (Corr.) (Rutaceae) .Medicinal plants are plants which used in number of ways because of the phytochemical constituents present in plants. Phytochemicals are the chemicals that present naturally in plants, now a day these phytochemicals more popular due to their countless medicinal use, Unlike pharmaceutical chemical these phytochemicals do not have any side effects. Anti-head lice activity of plant extracts are natural prevention for head lice mainly for women and children. Head lice resistance to chemical shampoos and pediculicides are increasing day by day although that are harmful to hair, hence anti-head lice activity of plants are needed for head lice treatment. These plants have a potential to prevent or kill the head lice similar to the action of synthetic shampoos.

KEYWORDS: *Azadirachta indica, Aegle marmelos*, Phytochemical, antilicicidal.

INTRODUCTION

Plants have been used for medicinal purposes long before recorded history. Primitive men observed appreciated the great diversity of plants available to them, the plants provide food, shelter and medicine. The plant that possess therapeutic properties or exert beneficial pharmacological effect on human body are generally designated as a medicinal plants. Medicinal plants naturally synthesize and accumulate some secondary metabolites like alkaloids, sterols, terpenoids, and flavonoids. Current research trends use variety of plant

extracts as alternative licicide and larvicides because they contain various phytochemicals. Phytochemicals are the chemical that present naturally in plants. Phytochemical play a vital role against number of disease such asthma, arthritis, cancer etc. Unlike pharmaceutical chemicals these phytochemicals do not have any side effects, since the phytochemicals cure disease without causing any harm to human beings. Also these phytochemicals have anti-head lice activity without harming other organisms and environment. Instead of using synthetic anti-head lice shampoo, use of plant extract is environment friendly.

Head lice resistance to chemical pediculicide are increasing, thus new alternative form or plant are needed for head lice treatment (Thamariselvi *et al.*, 2016). Hair hygiene is important to everyone in order to keep the hair clean and healthy. It promotes growth of hair, and prevents loss of hair, itching, infection, accumulation of dirt, dandruff, oil, stimulation of circulation by massage and brushing, shampooing, and combing are essential to maintain the hair clear and healthy, unclean hair containing dirt and excessive dandruff and sweat and sebum which will allow the growth of microorganisms and parasites on scalp (Soonwera, 2016).

Pediculosis is skin infection caused by hematophagous lice, the most common of which is head louse, *Pediculus humanus capitis* De Geer.; the infestations of human lice are prevalent worldwide, especially among school children in both developing countries. (Thamaraiselvi *et al.*, 2016).

The divine tree neem is mainly cultivated in the Indian subcontinent. In earlier centuries people introduced neem to West Africa from India. The Vedas called neem as ‘SARVA ROGA NIVARINI’ which means, one that cures all aliments and ills. Through history, neem tree had many fascinating aspects for the people of India. The neem tree was a symbol of health, in the sun and heat this evergreen tree was a heaven with its cooling shade. The medicinal property of the neem tree have been well known in India for over 4,000 years.

Aegle marmelos (Corr.) is commonly known as Bael (Sandeep *et al.*, 20110) or Bilava belonging to the family Rutaceae, it is slow growing, medium sized tree, up to 12 to 15 m tall with short trunk, thick, soft, flaking, bark, and spreading, sometimes spiny braches, fruit and leaf, and decoction of the bark have been used in traditional medicinal system for the treatment of various diseases and the tree is held scared by Hindus and offered in prayers to deities lord Shiva and Parvathi and thus the tree is also known as shivaduma. It is indigenous

to Indian subcontinent and mainly found in tropical and subtropical regions (Chakraborty *et al.*, 2012).

Chemical investigation on the products of neem tree was extensively undertaken in the middle of twentieth century. The neem tree is incredible plant that has been declared the tree of twenty-first century by the United Nations (Puri 1999).

The plant product or plant extract show an important role in diseases prevention and treatment through the enhancement of antioxidant activity, inhibition of bacterial growth, and modulation of genetic pathways. Neem tree and Bael tree has lot of phytochemical constituents and they are widely used in various purposes because of the phytochemical constituents present in plants. The present study is to analyses the phytochemical constituents of leaf extract of *Azadirachta indica* (A.Juss) and *Aegle marmelos* (Corr.). To evaluate the anti-head lice activity of *Azadirachta indica* (A.Juss) and *Aegle marmelos* (Corr.)(Leaves).

MATERIALS AND METHODS

Sample Collection

Fresh leaves of selected plant material such as *Azadirachta indica* (Meliaceae) and *Aegle marmelos* (Rutaceae) collected during September month. They were shade dried and ground to fine powder and stored in air tight container for further analysis.

Preliminary phytochemical analysis (Ramman, 2006, Karpagam*et al.,* 2008; Kokate*et al.,* 2001).

Extraction

The powdered plant leaves were collected and 15 g of it were measured and introduced in to 100 ml of ethanol, acetone, and water extraction carried out by shaker system for 48 hours. The nature and yield of the extract were noted. The extracts were stored in a refrigerator at 4℃ for further studied.

The extracts of selected plant leaves were tested for flavonoids, alkaloids, tannins, saponins, terpenoids carbohydrates, proteins, amino acids, steroids, glycosides, and resins, This phytochemical screening of extracts are carried out by standard methods (Ramman, 2006; Karpagam *et al.,* 2008; Kokate *et al.,* 2001).

1. Test for carbohydrates

To 2 ml of test solution adds two drops of the Molish reagent (a solution of α napthol in 95% ethanol). The solution is then poured slowly in to a test tube containing 2 ml of conc. Sulphuric acids so that two layers form. The formation of a purple product at the interface of the two layers indicates the presence of carbohydrate.

2. Test for proteins

It is used to determine the presence of peptide bonds in protein. To 3 ml of test sample is add 3% NaOH and a few drops of 1% $CuSO_4$. The solution turns from blue to violet (purple) or to pink. That indicates the presence of protein.

3. Test for starch

Mix 3 ml of test solution and few drops of dilute iodine solution. Blue color appears. It disappears on cooling and reappears on heating.

4. Test for amino acids

To 5ml of test sample solution is add a few drop of 40% NaOH and 10% of lead acetate and boiled the solution formation of black precipitate show the presence of amino acids.

5. Test for steroids

To 2 ml of extract and add 2 ml of chloroform and add 2 ml conc. Sulphuric acid. Shake well, chloroform one layer appear and acid layer show greenish yellow florescence which indicate the presence of steroids.

6. Test for glycosides

To the solution of extract add glacial acetic acid, few drops 5% ferric chloride and concentrated sulphuric acid are added, and observed for a reddish brown coloration at the junction of two layers and bluish green color in upper which indicates presence of glycosides.

7. Test for flavonoids

To 2 ml of extract and few drops of 1% of Ammonia solution. A yellow coloration is observed for the presence of flavonoids.

8. Test for alkaloids

To 0.5g of each extracts adds 5ml of 1% of aqueous hydrochloric acid and kept in water bath: 1ml of the filtrate is to be treated with Mayer's reagent (Potassium iodide). Formation of a yellow colored precipitate indicates the presence of alkaloids.

9. Test for tannins

To 0.5ml of extract solution, 1 ml of water and 1-2 drops of ferric chloride solution was added. Blue color was observed for gallic tannins and black color for catecholic tannins.

10. Test for saponins

To 1 ml of extract solution, 1 ml of water and shake it. Persistent foam indicates presence of saponins.

11. Test for terpenoids

2ml extract was mixed with 2ml of chloroform in a test tube. To this 3ml of conc. Sulphuric acid was added along the walls of the tube to form a layer. An interface with a reddish brown coloration confirmed the presence of terpenoids.

12. Test for gums

To 1 ml of extract add 3 ml of Dil. Hcl solution is added drop by drop till red coloration visualizes the presence of gums.

Anti-Head lice Activity

Sample collection

In the present study Neem and Bael leaves were collected from Kakkodi from Calicut. Students from IInd M.Sc. Botany chosen to provide us the head lice

Preparation of leaf extract

Fresh leaves of *Azadirachta indica* and *Aegle marmelos* were collected and extract is prepared using pestle and motor. Leaf extract and water poured in to clean Petri plate and labeled accordingly

Conduct of Experiment

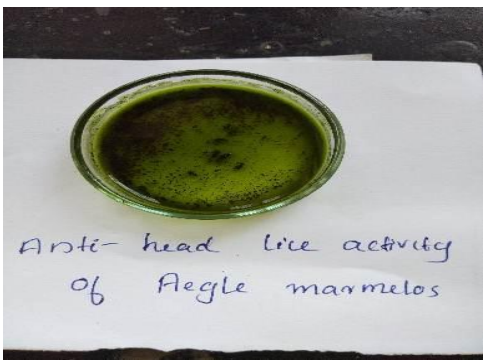
After collected specimen (Head lice, just before the experiment because head lice need human scalp for their survival, they can only live for 28 to 48 hours). The first petri plate contains without any treatment because the first would be controlled group. The second petri

plate contain neem leaf extract (without adding water). The third petri plate contains Bael leaf extract (without adding water) and the fourth petri plate contains commercially prepared anti-head lice shampoo (mediker). For control put cotton inside petri plates (without any treatment). Take clean 4 petri plates and add 5 head lice for each petri plates, after this add 2 ml of leaf extract (Neem and Bael) to two Petri plates and add 2 ml of diluted mediker shampoo to other petri plate contained 5 head lice. Observe petri plates after certain time intervels.

Experimental setup of ant-head lice activity *Azadiracht aindica*(A.Juss) and *Aegle marmelos* (Corr.) (Leaves)

***Azadirachta indica* leaves extract** **Mediker shampoo**

Control



***Aegle marmelos* leaves extract** **Mediker shampoo**

Control

RESULTS AND DISCUSSION

Table 1: Phytochemical analysis of *Azadirachta indica* (A.Juss)(Leaves).

Sl. No	Phytochemical Constituents	Acetone Extract	Ethanol Extract	Water extract
1	Alkaloid	+	+++	+++
2	Flavonoid	++	++	+++
3	Tannin	-	-	-
4	Terpenoid	-	-	+
5	Glycoside	-	-	+
6	Saponin	++	+++	++
7	Carbohydrate	+	++	++
8	Protein	+	+	+
9	Amino acid	+	+	+
10	Steroid	-	-	-
11	Gum	-	-	-

+ = Presence - = Absence

The above table 1 showed that phytochemical constituents present in *Azadirachta indica* Ethanol extract contain that alkaloid, saponin, flavonoid, amino acid, protein, carbohydrate. Acetone extract contain saponin, alkaloids, amino acids, protein, and carbohydrate. Water extract contains alkaloids, terpenoids, flavonoids, glycoside, amino acid, protein, Carbohydrate steroid, tannin and gum were completely absent in three extract.

These findings support the phytochemical analysis of leaves extract using Aqueous, Ethanol and Chloroform, reported by (Ramdass et al., 2012) The ethanol extract of *Azadirchtaindica*showed the presence of alkaloids, glycosides, flavonoids, soponins, tannins phenolic compounds and reducing sugar, alkaloids, flavonoids, glycosides, reducing sugar, tannins, saponins polysaccharides and phenolics were observed in chloroform extract of

Azadirachta indica but there was absence of terepenoides and phytosterols. Aqueous extract of *Azadirchtaindica* showed the presence of alkaloids, flavonoids, glycoside reducing sugar, polysaccharides and phenols but there was absence of tannins, saponins and terpenoids. Reducing sugar were found in *Azadirachta indica* in the solvent such as chloroform, ethanol, and aqueous (Ramadass*et al.*, 2012).

Table2: Phytochemical analysis of *Aegle marmelos* (Corr.)(Leaves).

Sl no	Phytochemical Constituent	Acetone Extract	Methanol Extract	Water extract
1	Alkaloid	++	+++	+++
2	Flavonoid	+++	-	+
3	Tannin	-	-	-
4	Glycoside	+	+++	+++
5	Saponin	+++	+	++
6	Terpenoid	-	+	+
7	Gum	-	-	-
8	Carbohydrate	+	++	+
9	Protein	+	+	+
10	Starch	-	+	++
11	Amino acid	+	+	+
12	Steroid	+	+	-

+ = Presence - = Absence

This table 2 shows that phytochemical constituents present in methanol leaf extract of *Aegle marmelos*, contain the presence of alkaloid, saponin, terpenoid, glycoside, steroid, amino acid, starch, protein. Acetone extract contain that the presence of saponin, alkaloid, flavonoid, glycoside, steroid, amino acid, carbo hydrate. Water extract contains alkaloids saponin, terpenoid, glycoside, amino acid, starch, protein, carbohydrate, Gum, tannin, were absent in three extract.

These observations support the phytochemical constituents of *Aegle marmelos* different extracts like ethanol, water, methanol chloroform, reveals the presence of phenols, tannins, saponins, terpenoids, flavonoids, so the *Aegle marmelos* leaf extract showed varying degree of medicinal properties like antibacterial, antifungal. They conclude that the presence of tannins, saponins, terpenoids and flavonoids seems to cause these activities (Saroj*et al.*, 2011).

These findings was also support, phytochemical constituents of different extracts of leaves contain alkaloids, terpenoids, tannins, flavonoids, glycosides, saponins, steroids, and it causes

antimicrobial activity and it increases the medicinal property of plants (Venkatesan*et al*., 2009). In this study presence of high amount of carbohydrate, alkaloids, proteins, glycoside, were obtained the phytochemical analysis of *Aegle marmelos.*

Table 3: Anti Head lice activity of *Azadirachta indica*(A.Juss)(Leaves).

Treatment	Time interval		
	1 hour	2 hour	3 hour
Neem leaf extract	4/5	5/5	5/5
Mediker synthetic shampoo	4/5	5/5	5/5
Control	5/5	5/5	5/5

From the table 3 shown that anti-head lice activity of *Azadirachta indica* leaf extract was similar to that mediker shampoo treatment. Neem extract treatment shows 4 head lice died over a period of 1 hour and remaining one head lice were died after observing one hour. Since it shown that 5 head lice died out of 5, which shows the anti-head lice activity of neem leaf extract, control there is no dead lice over 5 lice. In treatment with mediker shampoo 5 head lice were died after 3 hours. This results shows similar to that *Azadirachtaindica* shows anti-head lice activity against human head lice similar to synthetic mediker shampoo.

Thamaraiselvi *et al.,* 2016 reported that *Lawsoniainermis* extract showed the licicidal effect against *Pediculushumanuscapitis*, Same as *Lawsoniainermis* showed Neem leaf extract is highly beneficial for prevention of human head lice. In my study I used pure leaf extract of neem for anti-head lice activity, so we can directly or indirectly applied neem leaf extract to hair for prevention of head lice, Neem is highly used in many hair care products so it is not harmful for hair.

The *Annona squamosa* leaf extract was found to have pediculicidal effect potential against lice, results show that it was less effective compared with commercially prepared Licealiz because as the researchers observed the head lice live in just a span of few hours, the mortality increased but the treatment with the fastest pediculicidal effect was with Licealiz (Ballesteros *et al.*, 2014).

Table: 4 Anti-head lice activity of *Aegle marmelos,* (Corr.)(Leaves).

Treatment	Time interval		
	1 hour	2 hour	3 hour
Bael leaf extract	3/5	5/5	5/5
Mediker synthetic shampoo	4/5	5/5	5/5
Control	5/5	5/5	5/5

From the table 4 shown that anti-head lice activity of *Aegle marmelos* leaf extract was similar to that of mediker shampoo treatment. In *Aegle marmelos* treatment shows 3 head lice died over a period of 1 hour and remaining head lice were died after 2 hours. Since it shown that 5 head lice died out of 5, which shown that anti – head lice activity of Bael leaf extract. In control there is no dead lice over 5 lice. In treatment with mediker shampoo 5 head lice were died after 3 hours. This result shown that *Aegle marmelos* shows anti – head lice activity against human head lice similar to synthetic mediker shampoo.

CONCLUSION

Aegle marmelos is subtropical plant which is cultivated worldwide, it is native to India. Medicinal plants play a significant role in production of novel and valuable drugs in modern medicine.

Phytochemical analysis carried out in methanol extract of *Aegle marmelos*(Leaves), revealed the presence of alkaloid, saponin, terpenoid, glycoside, steroid, amino acid, starch, protein. Acetone extract revealed that the presence of saponin, alkaloid, flavonoid, glycoside, steroid, amino acid, carbohydrate. Water extract contains alkaloids, saponin, terpenoid, glycoside, amino acid, starch, protein, carbohydrate.

Phytochemical analysis carried out by the extract of *Azadirachta indica* ethanol extract reveled that alkaloid, saponin, flavonoid, amino acid, protein, carbohydrate, Acetone extract reveled that saponin, alkaloids, amino acids, protein, carbohydrate, Water extract contains alkaloids, terpenoids, flavonoids, glycoside, amino acid, protein, Carbohydrate. These phytochemicals present in these plants improve the medicinal and therapeutic properties. Both this plants have potential on becoming on effective drug product.

Hair hygiene is important to everyone, in order to keep hair clean and healthy. It promotes hair grow, The head lice *(Pediculushumanuscapitis*) is an obligate ectoparasite, which cause peduculosiscapitis, Pediculuscapitis is treated by removal of head lice by hand or by using synthetic shampoos, sometime synthetic shampoos cause scalp irritation, hair damage, so nontoxic alternative option are hence needed for head lice treatment, in the present study a new effort has been made for controlling the human head lice by applying the *Aegle marmelos* and *Azadirachta indica*. These plants have a potential to prevent or kill the head lice similar to the action of synthetic shampoos. Among this two plants *Azadirachta indica* is highly effective for head lice treatment, leaf powder of *Azadirachta indica* and *Aegle*

marmelos is used for natural head lice treatment. *Azadirachtaindica* and *Aegle marmelos* has complex of various phytochemical constituents can play a major role in pharmaceutical industry for drug preparation.

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