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Phytochemical screening and Antibacterial activity of Neem flower (*Azadirachta indica*) and Production of Homemade Soap

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Abstract: Neem tree (*Azadirachta indica*) belongs to the family Meliaceae. It is widely used in Indian subcontinent to treat many diseases without any side effects. Each part of the Neem tree has its specific medicinal value. Neem flower has a variety of uses including aromatherapy, cooking, cosmetics and health remedies. By analyzing the phytochemicals of Neem flower extract represents the presence of some secondary metabolites and this indicates the occurrence of antibacterial activity against gram positive and gram negative bacteria.

Keywords: *Azadirachta indica*, phytochemical analysis, antibacterial activity, homemade soap.

I. INTRODUCTION

Neem is an omnipotent tree and a sacred gift of nature. Neem tree is mainly cultured in the Indian Subcontinent. Botanical name of neem is *Azadirachtaindica*. The neem tree is an incredible plant that has been declared as a "Tree of 21st century" by United Nations. Neem is a large tree that is approximately 25 meters in height with a semi-straight Trunk. It is a flowering plant and normally starts fruiting after 3-5 years [1]. Different parts of the Neem plant contain numerous biologically active principles includes Azadirachtin, Meliacin: Gedunin, Salanin, Nimbin, Valassin, Quercetin, Nimbinin, Nimbidin and many other derivatives [2]. Numerous biological and pharmacological activities have been reported including antibacterial [3], antifungal [4] and anti-inflammatory activity. Extracts of Neem fruit, seeds, seed kernels, twigs, stem bark and root bark have been shown to possess repellent, anti-feedant, insect growth regulatory (IGR), anti ovipositional, fecundity and fitness reducing properties on insects [1]. In additional, Fruit, seeds, oil, leaves, root, bark and just about each part of the tree is bitter & contain compounds with verified anti-viral, anti-fungal, antiplasmiodal, antiseptic, antipyretic and anti-diabetic houses [5]. The tree is often covered in delicate flowers in the early summer. The flowers (white and fragrant) are arranged axillary, normally more-or-less drooping panicles which are up to 25 cm long [6]. It has a variety of uses, including aromatherapy, cooking, cosmetics and health remedies. The Neem flowers are dried, aged for months, fried & ingested to improve eyesight and treat digestive disorders, including excessive bile, phlegm and intestinal worm. The flowers are so traditionally used as an element tonic to the treatment of fever and nasal polyposis [**7**]. Extract from flower possess higher antioxidant activity [8]**.** Ethanolic extracts of flowers were found to possess greater free radical-scavenging activity [9]. Neem flower have antifertility activity. [10].Traditionally, Neem was used in Ayurveda for a number of conditions. Neem flower is used for Elimination of intestine worms, phlegm, bile suppression.

II. MATERIALS AND METHODS

1. Collection and Processing of Sample

The Neem flower (*Azadirachtaindica*) was collected from different Neem tress in the Erode local area, subjected to shade dry and it was crushed to powder and stored in air tight container.

2. Preparation of Extract

The powdered Neem flower was mixed with solvents (Water and Acetone) in the ratio of 1:10. Then the mixture was placed in rotator shaker for 24 hours and filtered. The filtrates were evaporated to dryness and crude obtained.

3. Phytochemical Screening

Water and Acetone extracts of Neem flower were subjected to qualitative tests for the identification of various active constituents.

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3.1. Test for Carbohydrates

(a) Molish's test: To 1 ml of extract, 2 ml of Molish's reagent is added. The mixture is shaken well and 2.0 ml of Concentrated Sulphuric Acid is added slowly along the sides of the test tube. A reddish ring formed indicates the presence of carbohydrates.

(b) Fehling's test: To a few drops of extract,2ml of Fehling's reagent is added. The mixture was shaken well and kept in a boiling water bath. A formation of brick red precipitate indicates the presence of sugar.

3.2. Test for Alkaloids

(a)Mayes's test: To a few drops of extract, two drops of Mayes's reagent is added by the side of the test tube. A green coloured precipitate confirms the test as positive.

(b)Wagner's test: To a few drops of extract, two drops of Wagner's reagent is added by the side of the test tube. A reddish brown coloured precipitate confirms the test as positive.

3.3. Test for Saponins

(a)Foam test: To a few ml of extract,20ml of distilled water was added in the test tube and the test tube is continuously shaken for 10 minutes. The formation of foam confirmed the presence of saponins.

(b)Froth test: To a few ml of extract, added the 20 ml of distilled water and shake for 15 mints, the formation of 1cm layer of foam to indicate the presence of saponins.

3.4. Test for Flavonoids: To a few ml of extract, few drops of diluted sulphuric acid is added. Orange colour develops which indicates the presence of flavonoids.

3.5. Test for Terpenoids: To 2 ml of extract, 2 ml of acetic anhydride and Concentrated Sulphuric Acid is added. Formation of green ring indicates the presence of terpenoids.

3.6. Test for Amino Acids: To a few drops of extract, few drop of Ninhydrin solution is added in a test tube. A characteristic blue colour indicates the presence of amino acids.

3.7. Test for Proteins: To 2 ml of extract, few drop of Millon's reagent is added. White precipitate indicates the presence of Proteins.

3.8.Test for Phlobotannins: Few drops of extract were boiled along with 1% Hydrochloric acid. Formation of red precipitate indicates the presence of phlobotannins.

3.9. Test for Coumarin: To 2 ml of extract, 10% of 3 ml Sodium Hydroxide is added. Formation of yellow indicates the presence of coumarin.

3.10. Test for Cycloglycosides: In a test tube added 5 ml of extract and 2 ml of acetic acid and 1 drop of ferric chloride and 1.0 ml of Concentrated Sulphuric Acid is added slowly along the sides of the test tube and allowed to stand. Formation of brown, violet, greenish rings indicate the presence of cycloglycosides.

3.11. Test for Quinone: Few drops of extract added 5 ml of Hydrochloric acid. Formation of yellow precipitate indicates the presence of quinone.

3.12. Test for Cholesterol: To 2 ml of extract, 2ml of chloroform and 2 ml of acetic anhydride is added. To this added 1 ml of Concentrated Sulphuric Acid. Formation of violet to blue green colour indicates the presence of cholesterol.

3.13. Test for Anthocyanins: To 2 ml of extract, 2 ml of 2N Ammonium chloride and ammonium is added. Appearance of pink red to blue violet colour indicates the presence of anthocyanins.

3.14. Test for Phenols: To 2 ml of extract, 3 ml of ethanol and a pinch of ferric chloride are added. A greenish yellow colour appears which indicates the presence of Phenols.

4. Antibacterial Assay

The bacterial sources required for Antibacterial activitywere collected from the Department of Biotechnology, Kongu Arts and Science College, Erode.

Agar well diffusion method was used to evaluate the antibacterial activity of Azadirachtin indicia extracts. Nutrient agar medium (pH 7.0) was used. It was seeded aseptically with 500 µl of freshly prepared inoculums (106 colony forming unit, CFU) and immediately mixed. For inoculam preparation, the colonies of bacteria such as ***E. coli*** and ***Bacillus subtilis*** were suspended in nutrient broth and turbidimetrically adjusted. Twenty five milliliters of seeded nutrient agar media was transferred into each Petri plate and solidify. The organisms were spreaded in different petri plates. Four wells were made in each plate. Test solution of 50 µl was poured into each respective well. These plates were incubated at 37ºC. After 24 hours of incubation, the diameter of the clear zones that showed inhibition of bacterial growth was measured in millimeter (mm). Experiment was done in triplicate and mean value of zone inhibition was calculated with standard error.

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2.5. BYPRODUCT

2.5.1. PREPARATION OF HOMEMEADE SOAP BY LYE METHOD

It deals with the making of home-made neem soap by natural method.

Caustic Lye

20g of Sodium hydroxide was taken and it was dissolved in 100ml of distilled water. Thoroughly mixed the solution until the Sodium hydroxide is dissolved. Kept this mixture for 24 hours to avoid the harmful effects of Sodium hydroxide

Preparation of soap

Well dried powder of neem flower was taken and it was made into paste using distilled water. Then it was mixed with the prepared lye and to this 100ml of coconut oil, 5ml of castor oil and 5ml of olive oil was added. For the preparation of soap, fat is necessary for saponification reaction. Here Castor Oil, Coconut Oil and Olive Oil acts as a fat source. Rose Oil was added for fragrance and additionally Vitamin-E capsules was added for Skin glowing. Mixed the above contents well and poured it in silicon moulds. To make the soap rigid, it was kept undisturbed for 24hrs. Finally the soap will be ready to use after 30days.

III. RESULT AND DISCUSSION

1. PHYTOCHEMICAL SCREENING OF NEEM FLOWER

The results of phytochemical screening for Neem flowers were shown in table 1 and fig. 1 and fig. 2

S.No	Test for Phytochemicals	Solvents	
		Aqueous	Acetone
1	**Carbohydrate** a. Molish's test b. Fehling's test	 + +	 + +
2	**Alkaloids** a. Mayer's test b. Wagner's test	 + +	 + +
3	**Saponins** a. Foam test b. Froth test	 - +	 + +
4	**Flavanoids**	+	-
5	**Terpenoids**	-	+
6	**Aminoacids**	-	-
7	**Test for Proteins**	-	+
8	**Phlobotannins**	-	+
9	**Coumarin**	+	+
10	**Cycloglycosides**	-	+
11	**Quinone**	-	+
12	**Cholestrol**	+	+
13	**Anthocyanin**	+	+
14	**Phenols**	+	+

Table 1: Phytochemical screening of Aqueous and acetone extract of Neem flower

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From the above results, acetone extracts have more phytochemicals when compared with aqueous extracts. So we conclude the acetone solvent has more capacity to extract the secondary metabolites. The phytochemicals present in both extracts are Carbohydrates, alkaloids, saponins, coumarin, cholesterol, anthocyanin and phenol.

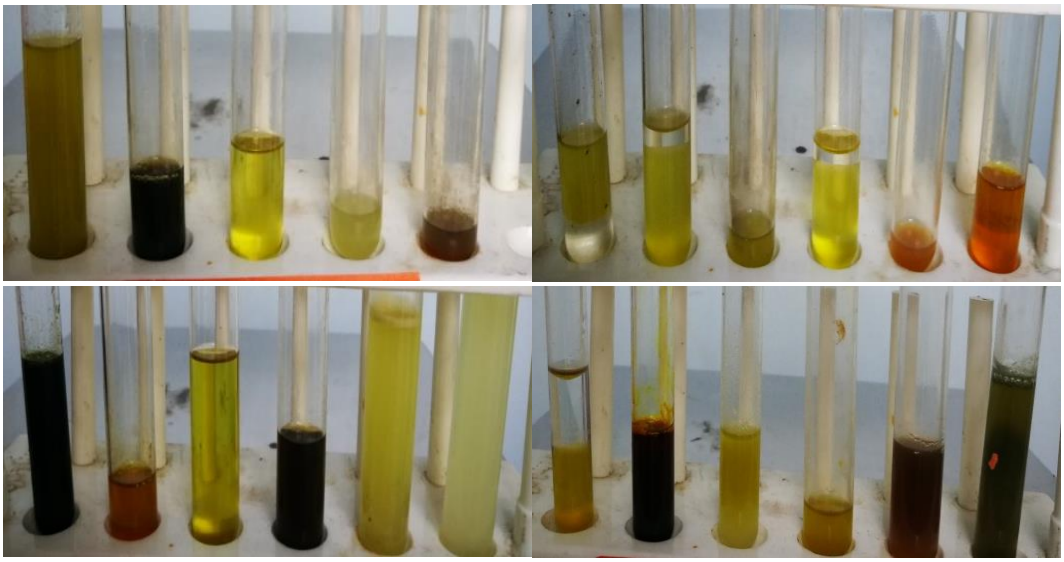

Fig. 1: Results showing the phytochemical screening of Aqueous flower extract of *Azadirachta indica*

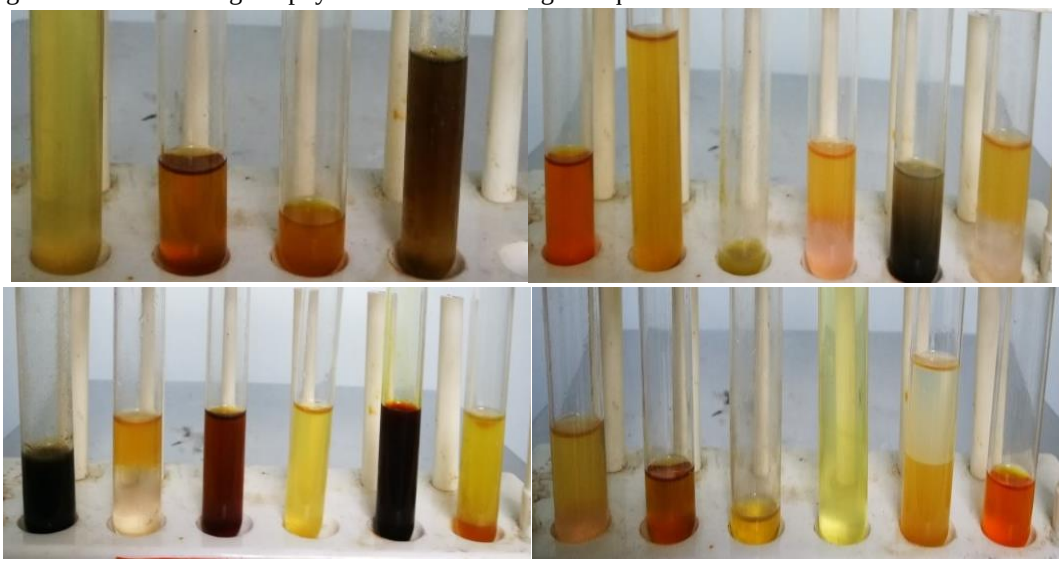

Fig. 2: Results showing the phytochemical screening of Acetone flower extract of *Azadirachta indica*

2 ANTIBACTERIAL ASSAY OF NEEM FLOWER

The extract of Neem flower had been tested for their antibacterial activities and an interesting antibacterial profile has been observed against gram positive (*Bacillus subtilis*) and gram negative bacteria (*Escherichia coli*). The Neem flower extract showed enormous activity against all two bacteria tested. The activities of extracts are mentioned in the terms of zones of inhibitions (mm).

S. NO	EXTRACTS	ZONE OF INHIBITION (mm)	
		Bacillus subtilis	***Escherichia coli***
1.	Aqueous	1±0.1	15±0.3
2.	Acetone	18±0.2	7±0.2

TABLE 2: Antibacterial activity of Aqueous and Acetone extract of Neem flower

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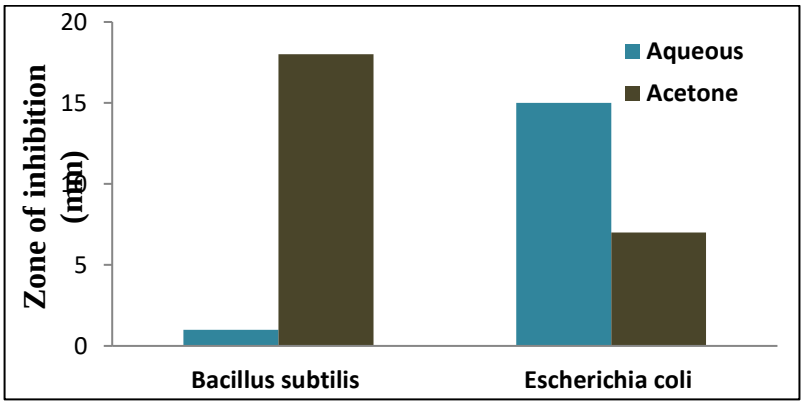

Fig. 3: Results of antibacterial activity Aqueous and Acetone extracts

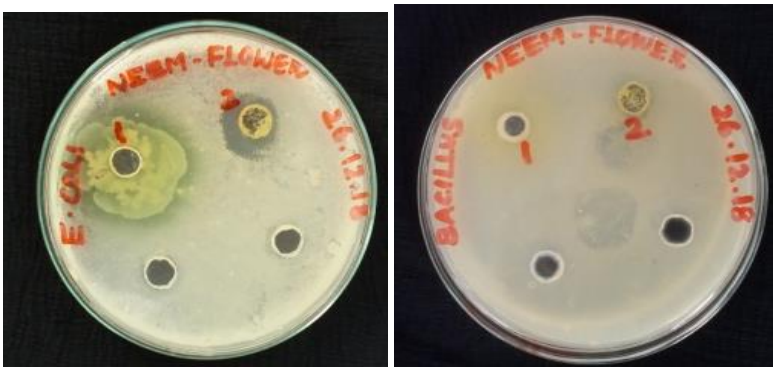

Fig. 4: Results of antibacterial activity Aqueous and Acetone extracts

The diameter of inhibition zones against *Bacillus subtilis* was 1mm and 18mm for aqueous and acetone extracts of Neem flower respectively. The diameter of inhibition zones (DIZ) against *Escherichia coli* was 15mm and 7mm for aqueous and acetone extracts of Neem flower respectively.

3. BY-PRODUCT

Neem flower have a variety of uses, including aromatherapy, cosmetics, health remedies and cooking. Organic homemade Neem soap without any chemical ingredients was made by using different Neem flower extracts.

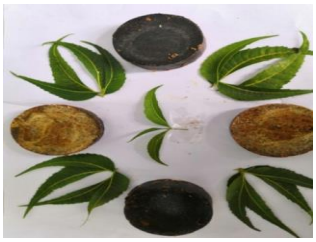

Fig. 5: Shows the production of homemade Neem soap from flower of *Azadirachta indica*

IV. CONCLUSION

The different parts of neem tree contain various active compounds which are rich in antibacterial activity. The present study highlights the phytochemical analysis of neem flowers. Various bioactive compounds like alkaloids flavonoids, coumarin, leucoanthocyanin etc., were present in aqueous and acetone extract. A true soap need to cleanse the body properly without disturbing the pH level of skin. Our work reveals the preparation of homemade neem flower soap, neem flowers are rich in cholesterol so the soap prepared by these neem flower contains sufficient fat content when compared to the soaps prepared by other neem components (leaf, flower, unripened and ripened fruit and seed). As per the results and discussion of the present study, flowers of Neem plant contain antibacterial activity, so preparing the soap using neem components destroys the microorganism which keeps our skin safe and healthy. So the homemade neem soap can be replaced with other synthetic soaps for better results.

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