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RESEARCH ARTICLE

PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF NEEM SEED (*AZADIRACHTA INDICA*) AND PRODUCTION OF HOMEMADE SOAP

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ABSTRACT

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 seed. Various bioactive compounds like alkaloids, flavonoids, coumarin, leucoanthocyanin etc., were present in aqueous and acetone extract of neem seeds. A soap must cleanse the body properly without disturbing thepH level of the skin. As per the results and discussion of the present study, neem seeds contain antibacterial activity and it also has appreciable quantity of oil. So preparing the soap using neem seed destroys the microorganism which keeps our skin safe and healthy. The homemade neem soap can be replaced with other synthetic soaps for better results

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INTRODUCTION

Neem is mainly cultured in Indian subcontinent. Which is considered as a sacred gift of nature, it is a kind of omnipotent tree. It is referred as Azadirachtaindica (A.indica) botanically. United Nations declared this incredible plant as a "Tree of 21st century" (16). Neem tree has numerous biological and neurological activities include antibacterial (22), antifungal (2) and anti-inflammatory activity. It is a flowering plant and it starts fruiting after 3-5 years, it grows approximately up to 25 meters and has semi-straight trunk (33).It has gained the distinction of being the most researched tree in the World. Neem product extracts shows repellent, anti-feed ant, insect growth regulatory (IGR), and fitness reducing properties on insects (33). In addition to these activity the neem products are bitter and has compounds with verified anti-viral, anti-fungal, antispasmodic, antiseptic, antipyretic and anti-diabetic activities (25). In about 10 years the tree becomes fully productive. It can produce up to 50 kg of fruits annually, after attaining tenth years and onwards. (19).Neem seed are bactericidial against gram negative and gram positive pathogens, and thus have a broad spectrum activity; they also have a synergistic interaction in combination with antibiotics (37).

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There by scientists reveal that neem seed weighs average of 0.28g, which of 50.89% kernel and 49.11% hull. It contains 29.27% of lipids, 12.10% of protein and 43.28% of parietal constituent (43).

MATERIALS AND METHODS

The present study mainly deals with the analysisof phytochemical constituents and antibacterial capacity found in seed of *Azadirachtaindica* and broadly evaluate its biochemical constituents, element composition and some secondary metabolites using standard protocols as follows.

COLLECTION AND PROCESSING OF SAMPLE

The Neem seed (*Azadirachtaindica*) was collected from different neem tress in the Erode local area and it was washed separately to eliminate dust and other foreign particles and subjected to shade drying for about 15 to 20 days. The dried neem products as further crushed to powder using mixer or blender and the powder was stored in air tight container.

PREPARATION OF EXTRACT: The powdered neem seed (*Azadirachtaindica*) was mixed with solvents (Water, Acetone) in the ratio of 1:10 (gram: millilitre). The mixture was placed in 250ml conical flask with Teflon lids. The flask was placed in rotator shaker for 24 hours.

After incubation, the mixture was filtered through Whatmann No.1 filter paper. The filtrates were evaporated to dryness and crude obtained was stored at $5^{o}C$ in refrigerator until further analysis. All these extracts were used for the identification of active constituents by following analysis.

PRELIMINARY PHYTOCHEMICAL SCRENNING: Water and Acetone extracts of neem seed were subjected to qualitative tests for the identification of various active constituents like alkaloids, flavonoids, steroids, glycosides, phenols, terpenoids, tannins, carbohydrates, proteins, fats, etc.

Test for Carbohydrates

- **Molish's test:** To a few drops of extract, 2 ml of Molish 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 and allowed to stand. A reddish ring formed at the junction of two solutions indicates the presence of carbohydrates.
- **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 for five minutes. A formation of brick red precipitate indicates the presence of sugar.

Test for Alkaloids

- **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.
- **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.

Test for Saponins

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

Test for Tannins

Lead Acetate Test: To a few ml of extract, add few drops of 1% lead acetate. The mixture is shaken well. A yellowish precipitate indicates the presence tannins.

Test for Flavonoids

Acid test: To a few ml of extract, few drops of diluted sulphuric acid is added. Orange colour develops which indicates the presence of flavonoids.

Test for Terpenoids

Acetic anhydride test: To 2 ml of extract, 2 ml of acetic anhydride and concentrated SULPHURIC ACID is added. Formation of blue, green rings indicate the presence of terpenoids.

Test for Amino Acids

Ninhydrin test: 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.

Test for Proteins

Biuret Test: Test solution was treated with 10% sodium hydroxide solution and two drops of 0.1% copper sulphate solution and observed for the formation of violet/pink colour.
Millon's test: To a few ml of extract, few drop of Millon's reagent is added. White precipitate indicates the presence of Proteins.

Test for Glycosides

Libermann's test: To 2 ml of extract, 2ml of chloroform and 2 ml of acetic anhydride is added. Formation of violet to blue to green reddish brown ring indicates the presence of glycosides.

Test for Cardiac Glycosides

In a test tube added 5 ml of extract and 2 ml of glacial acetic acid and 1 drop of ferric chloride and 1.0 ml of concentratedsulphuric 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 cardiac glycosides.

Test for Phlobotannins

Few drops of extract is boiled along with 1% Hydrochloric acid. Formation of red precipitate indicates the presence of phlobotannins.

Test for Coumarin

To 2 ml of extract, 10% of 3 ml sodium hydroxide is added. Formation of yellow indicates the presence of coumarin.

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 sulphuricacidis added slowly along the sides of the test tube and allowed to stand. Formation of brown, violet, greenish rings indicate the presence of cycloglycosides.

Test for Total Phenols

To 2 ml of extract, 3% of Ferric chloride is added. Formation of deep blue colour indicates the presence of total phenol.

Test for Quinone

To few drops of extract 5 ml of Hydrochloric acid is added. Formation of yellow precipitate indicates the presence of quinone.

Test for Anthraquinones

To 2 ml of extract, 2 ml of 10% Ammonium hydroxide is added. Formation of bright pink colour indicates the presence of anthraquinones.

Test for Steroids: To 2 ml of extract, 2ml of chloroform and 2 ml of acetic anhydride is added reddish brown colour is formed. To this added 1 ml of concentrated sulphuricacid. Formation of violet to blue green colour indicates the presence of Steroids.

Test for Carotenoids: 10 ml of extract is evaporated to dryness. To these 2 to 3 drops of concentrated sulphuricacidand chloroform was added. Formation of blue colour indicates the presence of carotenoids.

Test for Fatty acids: To a few ml of extract are pressed in filter paper and dried. The transparency appeared in the filter paper indicates the presence of fatty acid.

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.

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.

Test for Leuco Anthocyanin: To 5 ml of extract, 5 ml of Isoamyl alcohol is added. Formation of upper layer red indicates the presence of leucoanthocyanin.

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.

Antibacterial assay by agar well diffusion method

Antibacterial activity of *Azadirachta indicia*seed was determined by disc diffusion method. The bacterial sources were collected from the Department of Biotechnology, Kongu Arts and Science College, Erode.

Procedure: Agar well diffusion method was used to evaluate the antibacterial activity of *Azadirachtaindica* extracts against the test microorganism. Nutrient agar medium (pH 7.0) was prepared and autoclaved. It was allowed to cool up and then 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 solidified. 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 37^{o}C. After 24 hours of incubation, the diameter of the clear zones that showed inhibition of bacterial growth and it was measured in millimeter (mm). Experiment was done in triplicate and mean value of zone of inhibition was calculated with standard error.

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. Stirred it well until Sodium hydroxide is completely dissolved.Kept this mixture for 24hrs to avoid the harmful effects of Sodium hydroxide.

Preparation of soap: Well dried powder of neem seed 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 improving the glowing nature of skin. 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.

RESULTS AND DISCUSSION

Phytochemical screening of neem seed: The results of phytochemical screening for neem seeds were shown in table5.5.1 and figure5.5.1A and 5.5.1B.

TABLE 5.1 Phytochemical screening of Aqueous and acetone extract of Neem Seed: The above table shows the phytochemical screening of neemseed extract. It shows the presence of certain important components such as

- Carbohydrates, saponins, proteins, phenols in water extract.
- Carbohydrates, alkaloids, saponins, flavanoids, protein, coumarin, quinone, phenols in acetone extract.

ANTIBACTERIAL ASSAY OF NEEM SEED: The extract of neem seed 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 seed extract showed enormous activity against all two bacteria tested. The activities of extracts are mentioned in the terms of zones of inhibitions (mm).

The diameter of inhibition zones (DIZ) against*Bacillus subtilis* was 1mm and 2mm for aqueous and acetone extracts of neem seed respectively. The diameter of inhibition zones (DIZ) against *Escherichia coli* was 20mm and 2mm for aqueous and acetone extracts of neem seed respectively. Neem seed contains antimicrobial activity (Natarajan V et al, 2003)

BY-PRODUCT: Neem seed have a variety of uses, including aromatherapy, cosmetics, health remedies,oil preperation and cooking (Uses of Neem seed, MIKE ANDREW). An organic homemade neem soap without any chemical ingredients was made by using different neem seed extracts. The neem seed kernel has appreciable quantity of oil and can be used in production of soap.(Idongesit Effiong Sampson, October2018)

S.NO	Test for Phytochemicals	Solvents	
		Aqueous	Acetone
1.	Carbohydrate Molish's test Fehling's test	- +	- +
2.	Alkaloids a.Mayer's test b. Wagner's test	- -	+ +
3.	Saponins a. Foam test b. Froth test	- +	+ +
4.	Flavanoids – Acid test	-	+
5.	Terpenoids – Acetic anhydride test	-	-
6.	Aminoacids – Nihydrin test	-	-
7.	Test for Proteins – Millon's test	+	+
8.	Glycosides – Libermann's test	-	-
9.	Totalphenols	-	-
10.	Steroids	-	-
11.	Phlobotannins	-	-
12.	Coumarin	-	+
13.	Cycloglycosides	-	-
14.	Quinone	-	+
15.	Anthroquinones	-	-
16.	Carotenoids	-	-
17.	Fatty Acids	-	-
18.	Cholestrol	-	-
19.	Anthocyanin	-	-
20.	Leuco Anthocyanin	-	-
21.	Phenols	+	+

Figure 5.1A Results showing the phytochemical screening of Aqueous Seed extract of *Azadirachta indica*

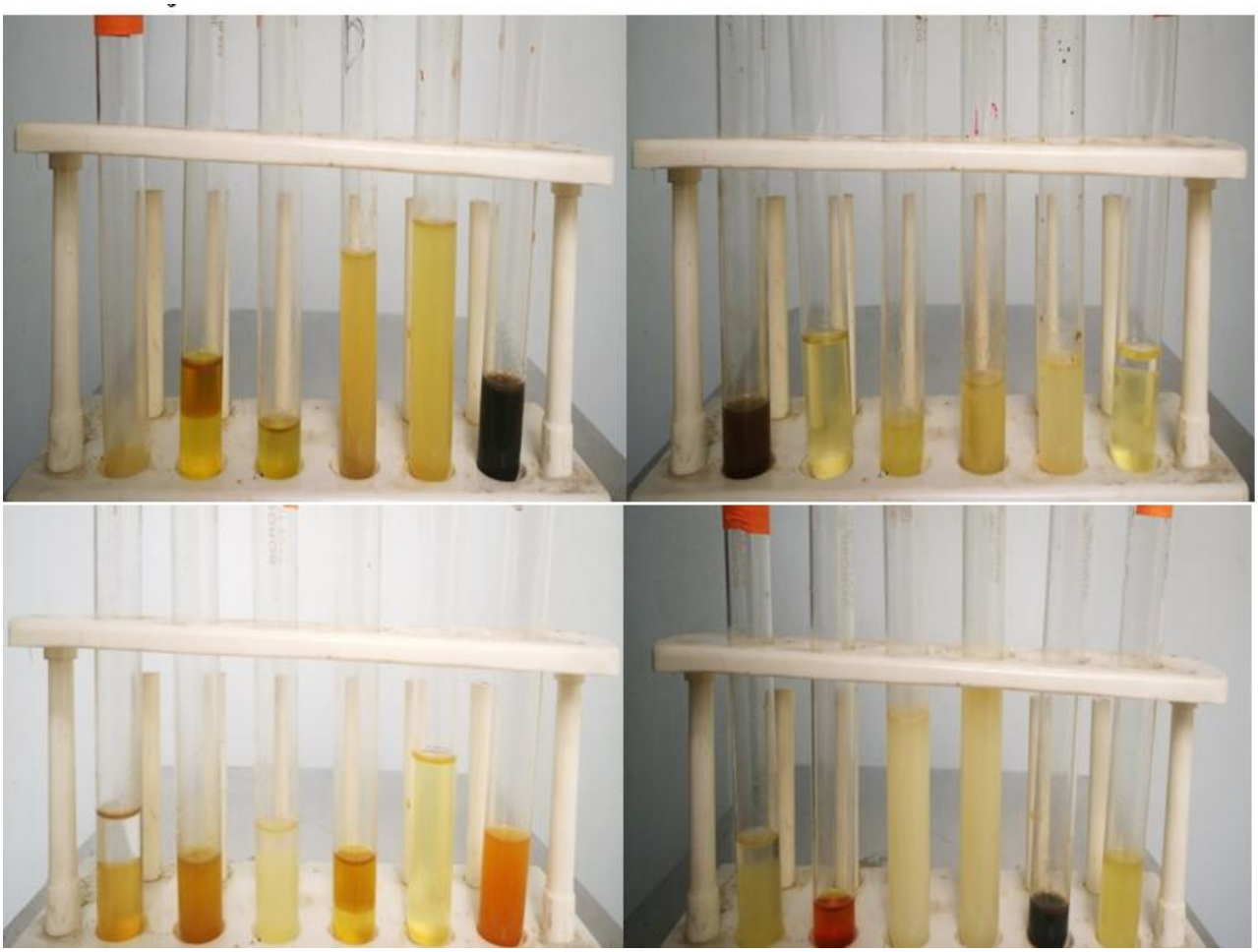

Figure 5.1 B Results showing the phytochemical screening of Acetone Seed extract of *Azadirachta indica*

S.NO	EXTRACTS	ZONE OF INHIBITION (mm)	
		Bacillus subtilis	Escherichia coli
1.	Aqueous	1 ± 0.2	20 ± 0.1
2.	Acetone	2 ± 0.3	2 ± 0.2

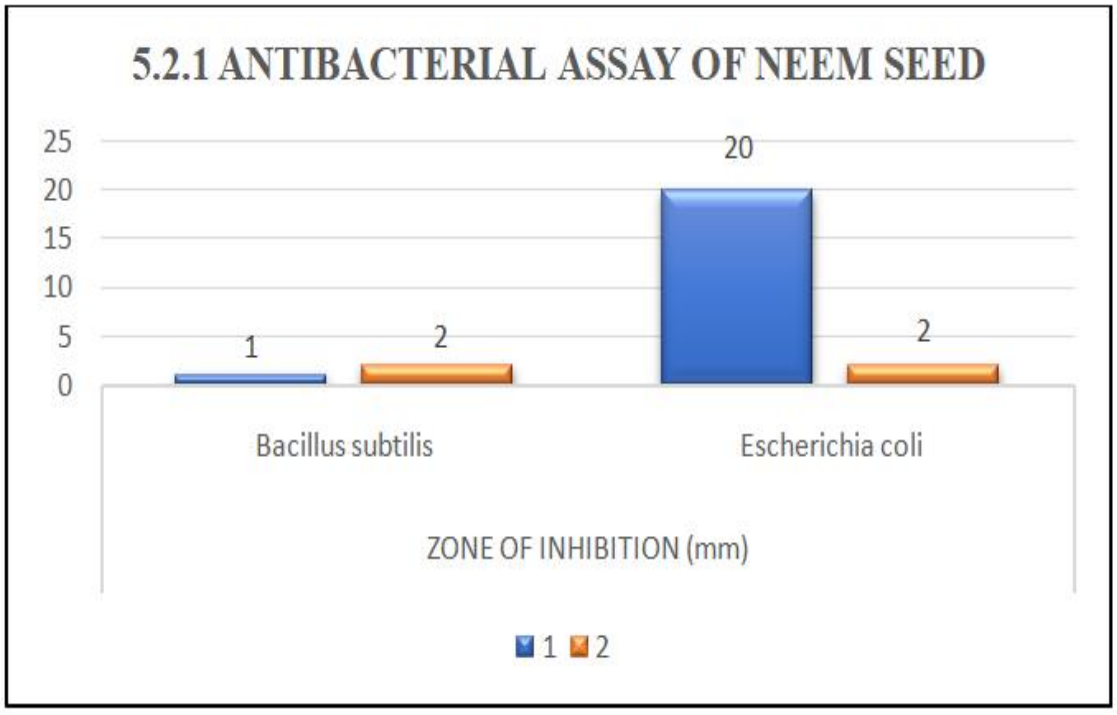

Figure 6.1.

Figure 6.2.

Conclusion

The different parts of neem tree contain various active compounds which are rich in antibacterial activity. The extracts from the seeds of neem tree is known for its use by traditional medical practitioners.The neem seed kernel has appreciable quantity of oil and can be used in production of soap (43). The present study highlights the phytochemical analysis of neem seed. Various bioactive compounds like alkaloids flavonoids, coumarin, Leuco-anthocyanin etc., were present in aqueous and acetone extract. A true soap need to cleanse the body properly without disturbing the pH level of skin. The soap need to be with foaming and cleansing activity. Our work reveals the preparation of homemade neem seedsoap, as per the results and discussion of the present study neem seed contain antibacterial activity, so preparing the soap using neem seed 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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4. AlokMaithaniet al. / Journal of Pharmacy Research 2011,4(6),1824-1827 1824-1827 Review Article ISSN: 0974-6943 Available online through http://jprsolutions.info *Corresponding author. Versha Parcha Dept. of Pharmaceutical Sciences, SBS (PG) Institute of Bio-Medical Sciences Research, Balawala, Dehradun, India INTRODUCTION AZADIRACHTA iNDICA (NEEM) LEAF: A REVIEW Alok Maithani1 , VershaParcha*2, Geeta Pant3 , Ishan Dhulia2 , and Deepak Kumar2. 1. Dept. of Pharmaceutical Chemistry, GRD(PG) Institute of Management and Technology, Rajpur, Dehradun. 2. Dept. of Pharmaceutical Sciences,SBS (PG) Institute of Bio-Medical Sciences & Research, Balawala, Dehradun. 3. Dept. of Chemistry, Birla Parisar, HNBGU, Srinagar. Received on: 11-02-2011; Revised on: 16-03-2011; Accepted on:21-04-2011
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