PHYTOCHEMICAL SCREENING AND THE ANTIMICROBIAL ACTIVITY OF THE LEAVES OF AZADIRACHTA INDICA, A. JUSS.

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ABSTRACT *The present study includes the phytochemical detection and antimicrobial activity of the leaves of Azadirachta indica, A. Juss. Phytochemical screening of the leaf extract of leaves both fresh and shade dried indicated the presence of flavonoids, terpenoids, tannins, alkaloids, steroids and glycosides. The antibacterial activity of methanol, ethanol and aqueous extracts of leaves of A. indica was evaluated against the human pathogenic bacteria like Staphylococcus aureus and Pseudomonas aeruginosa by well diffusion method. Among the extracts analysed ethanolic extracts showed promising results whereas aqueous extract did not show any zone of inhibition. The ethanol leaf extract (200µg/ml) showed maximum inhibition against Pseudomonas aeruginosa (16mm) and Staphylococcus aureus (16mm). Phytochemical tests carried out showed that the antibacterial activity of plant Azadirachta indica leaves was due to the presence of phytochemical compounds present in it.*

Keywords: *Azadirachta indica*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, antibacterial activity.

I. Introduction

Azadirachta indica (Neem) is one of the most useful traditional medicinal plant. Every part of the tree has been used as traditional medicine for household remedy against various human ailments. Most of the parts of the plant contain compounds with proven antiseptic, antiviral, antipyretic, anti-inflammatory, antiulcer and antifungal properties [1]. Plant parts like root, bark, seed and leaves have been an important source of medicine since thousands of years. In recent years a predominant interest has been observed in evaluating different plant extracts for their antimicrobial properties against bacteria [2]. The chemical constituents found in the leaves of neem are nimbin, nimbanene, 6-desacetylnimbinene, nimbandiol, nimbolide, ascorbic acid, n-hexacosanol and amino acid, 7-desacetyl-7-benzoylazadiradione, 7-desacetyl-7-benzoylgedunin, 17-hydroxyazadiradione and nimbiol [3]. Neem contains different active phytoconstituents such as alkaloids, glycosides, trepenoids, steroids and tannins[4]. Neem leaf is effective in treating eczema, ringworm, acne, anti-inflammatory, antiheperglycemic properties and it is used to heal chronic wounds , diabetic food and gangrene developing conditions. It is believed to remove toxins from the body, neutralize free radicals and purify the blood. It is used as anticancer agent and it has hepato-renal protective activity and hypolipidemic effects[5].

A wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, etc. which have been found *in vitro* to posses medicinal properties. Pharmacological studies have accepted the value of medicinal plants as potential source of bioactive compounds [6] Phytochemicals from medicinal plants serve as lead compounds in antimicrobial discovery [7] and [8]. The present study was aimed to evaluate phytochemical screening and the antibacterial potential of leaves of *Azadirachta indica* against bacterial pathogens.

2. Materials and Methods

2.1: Collection of raw materials and preparation of extracts

The leaves of *Azadirachta indica*, A. Juss. were collected locally and authenticated. Fresh and shade dried leaves were used for the study. The leaves were cleaned. One portion of the leaves were shade dried for one week and pulverized to coarse powder. For the preparation of the ethanolic, methanolic and aqueous extract, 1g of the dried ground plant material was weighed and extract was prepared by refluxing with 10ml of the solvent. The extract was filtered and collected. The extract was then wrapped with aluminium foil to prevent evaporation. Extracts of leaves were stored in air tight containers at 4^0 C for various procedures.

2.2 Phytochemical Screening of extracts

Ethanol methanol and aqueous extracts were used for preliminary phytochemical screening using standard procedures [9].

2.3 Determination of Anti bacterial activity

2.3.1 Preparation of Culture Medium

Nutrient agar medium

1000ml of distilled water was boiled in a round bottom flask and 5gm of NaCl, 20gm agar,10gm meat extract and peptone were added by continuous stirring. The mouth of the flask was wrapped with aluminium foil and tied tightly. The medium was sterilized at a pressure of 15 lbs and 121^{0}C for 15 minutes in an autoclave. The pH of the Nutrient agar medium was adjusted to 7.4 (at room temperature after gelling).

Microorganisms used

The bacterial strains were procured from the Cambrit Biosolution, Ernakulam were used for the present study. They include *Staphylococcus aureus* (Gram positive) and *Pseudomonas aeruginosa* (Gram negative). The bacterial strains were maintained in Muller Hinton Agar (MHA, pH 7.2) at 37±1^{0}C .The stock culture slants were maintained at 4^{0}C.

Well Diffusion Method

The antibacterial activity of the leaf extracts was determined using agar well diffusion method by following the following procedure. Nutrient agar was inoculated with the given microorganisms *Staphylococcus aureus* and *Pseudomonas aeruginosa*, by spreading the bacterial inoculums on the media. Wells were punched in the agar and filled with plant extracts. Control wells containing neat solvents (negative control) were also run parallel in the same plate. The plates were incubated at 37°C for 18 hours and the antibacterial activity was analysed by measuring the diameter of the zone of inhibition. The antibacterial potential of the different extracts was evaluated by comparing their zones of inhibition (10).

3. Results and Discussion

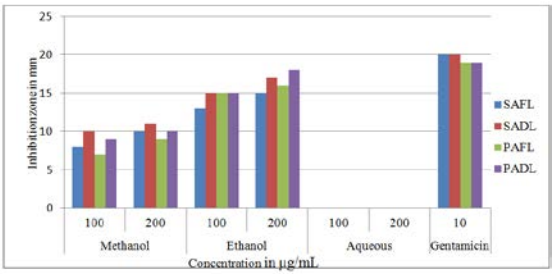
Neem and its ingredients play role in the inhibition of growth of numerous microbes such as viruses, bacteria, and pathogenic fungi. The role of neem in the prevention of microbial growth has been reported[11] . The results of phytochemicals in the present investigation showed that the plant leaves both fresh and sun dried contained alkaloids, flavanoids, resins, bitter, tannins, cardiac glycosides, reducing sugar and triterpenes, volatile oils, saponins and steroids in the ethnol extracts. (Table 3.1).

Table 3.1 Phytochemical screening of the fresh and shade dried leaves of *Azadirachta indica*

chemical constituents	Fresh leaves			Shade dried leaves		
	ethanol	methanol	water	ethanol	methanol	water
Alkaloids	+	+	-	+	+	-
Flavonoids	+	-	-	+	-	-
Resins	+	-	-	+	-	-
Bitter	+	-	-	+	-	-
Saponins	+	-	-	+	-	-
Tannins	+	-	-	+	-	-
Steroids	+	-	-	+	-	-
Cardiac glycosides	+	+	+	+	+	+
Reducing sugars	+	+	-	+	+	-
Volatile oils	+	-	-	+	-	-

There has been reports of the presence of different phytochemicals with biological activity that has valuable therapeutic index [12, 13]. It has been observed that the biologically active phytochemicals were present in the ethanolic extracts of *Azadiracta indica*. *Staphylococcus aureus* a gram-positive, round-shaped bacterium found in the nose, respiratory tract, and on the skin, common cause of skin infections [14] *Pseudomonas aeruginosa* a common Gram-negative, rod-shaped bacterium that can cause disease in plants and a species of considerable medical importance, *P. aeruginosa* is a multidrug resistant pathogen recognised for its ubiquity [15]. In the present study, the antibacterial activity of the leaves of A. *indica* was tested by the disc diffusion method against two bacterial species *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The results of the present investigation showed that the ethanol extracts of the fresh and shade dried leaves at concentration of 200µL/disc, gave various inhibition zone against s*taphylococcus aureus* (Gram-positive species) as well as *Pseudomonas aeruginosa* the Gram-negative bacteria (Table 3.2). The shade dried leaves showed higher zone of inhibition (17mm in *Staphylococcus aureus* and 18 mm in *Pseudomonas aeruginosa* at 200µL/disc). The methanolic extracts showed a decreased zone of inhibition when compared to the ethanol extracts (11mm in *Staphylococcus aureus* and 10 mm in *Pseudomonas aeruginosa* at 200µL/disc) Aqueous extracts did not record any anti microbial activity.

Fig 3.1: Antimicrobial activity of fresh and dried leaf extracts of *Azadirachta indica*,A. Juss



SAFL- *Staphylococcus aureus* on fresh leaves
SADL- *Staphylococcus aureus* on shade dried leaves
PAFL- *Pseudomonas aeruginosa* on fresh leaves
PADL- *Pseudomonas aeruginosa* on shade dried leaves

The presence of these phytochemical components may be responsible for the observed antimicrobial activity of the plant leaf extract. This findings conforms to the report of [16] in which similar constituents was found to exhibits antiprotozoal and antibacterial activities. Antibiotic resistance is a major concern and development of new agents from plants could be useful in meeting the demand for new antimicrobial agents with improved safety and efficacy (17). Gentamycin was used as positive control and ethanol and water were used as negative control in the present investigation. All test strains of bacteria were found to be sensitive to Gentamycin. The solvent either ethanol or water were used as the negative control which did not show any zone of inhibition against tested bacteria. Results of the agar well diffusion method are shown in Fig 3.1. Leaf extract exhibited antibacterial activity against all the tested bacteria at all concentrations, where as aqueous extract does not showed any zone of inhibition. By comparing zone of inhibition exhibited by Gentamycin, in *Azadirachta indica* fresh and shade dried leaves ethanolic extract showed a considerable zone of inhibition (20mm in *Staphylococcus aureus* and 19 mm in *Pseudomonas aeruginosa* at 20 µL/disc). and therefore the leaf extract can be performed as a substituent for Gentamycin.

4. CONCLUSION

From the present study, it was concluded that the crude ethanol leaf extracts of *Azadirachta indica* have great potential as an antibacterial agent. The outcome of this work has shown that the leaf extracts both fresh and shade dried of *Azadirachta indica* against two bacterial strains. The antibacterial properties of these extracts may be due to the presence of above mentioned phytochemicals detected in the ethanolic extracts during the phytochemical screening. The presence of a number of chemical compounds may be attributed to the medicinal properties of this plant. The results of this analysis support the traditional use of *Azadirachta indica* leaves as an antibacterial agent and for treating skin related disorders. The isolation and structural elucidation of the bioactive compounds responsible for the observed pharmacological activities is to be carried out.

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