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Phytochemical and Bioassay Screening for the Fruits of Azadirachta Indica

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Article Details

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ABSTRACT

The MeOH and aqueous Ext of A. indica fruits were found active against P. aeruginosa. The results of methanolic extract and Aq Ext showed good antibacterial activity against S. aurous while other showed moderate to low activity against the tested pathogens. The results showed that A. indica has good antifungal activity against F. oxysporum and A. niger and low activity against P. notatum. The crude extract and Aq Ext, showed low activity against the tested insects except MeOH and aqueous Ext. From the results it may be concluded that A. indica shows low insecticidal property. The phytotoxic results of crude extract and Aq Ext of A. indica against Lemna minor. The methanolic extract showed significant result in growth inhibition at concentrations of 1000 and 100 µg/ml, respectively. While no phytotoxic activity was observed at concentration 10 µg/ml. Paraquat was used as standard plant growth inhibitor. The results showed that MeOH Ext and Aq Ext of the A. indica fruits showed negative hemagglutination activity against human RBCs of all blood groups.

1.1 Introduction

Neem (Azadirachta indica) is a member of the Meliaceae family and its role as health promoting effect is attributed because it is rich source of antioxidant. It has been widely used in Chinese, and Unani medicines world-wide and Subcontinent in the treatment and prevention of various diseases [1]. Biological activities attributed to different parts and extracts of A. indica plant include antiplasmodial, antioxidant, anticancer, antibacterial, antiviral, larvicidal and fungicidal activities [2]. There was a significant inhibition of tumour load, in both systemic tumour model studies. The incidence of the plant is also reduced by both doses of Azadirachta indica extract [3].

Infections usually occur in the mouth (oral thrush), vagina (vulvovaginitis), nails (paronychia) and skin, warm body parts such as the axillae, intergluteal folds, groin, or inframammary folds [4]. A. indica has been found to be effective in treating a number of ailments, including bacterial infections. A. indica is a rich source of antimicrobial agents [5]. Neem has been called "an amazing tree" and "a natural drug store." All parts of the plant, especially the leaves, bark, seed oil and their purified products are widely used in cancer treatment. More than 60 different types of organic chemicals including terpenoids and refined steroids in this plant [6]. The extract from bark, leaves, fruits and root have been used to control leprosy, intestinal helminthiasis and respiratory disorders in children [7]. Neem tree has adaptability to a wide range of climatic, topographic and edaphic factors. It thrives well in dry, stony shallow soils and even on soils having hard calcareous or clay pan, at a shallow depth. Neem tree requires little water and plenty of sunlight [8]. The world health organization estimates that 80% of the population living in the developing countries relies exclusively on traditional medicine for their primary health care [9]. Traditionally different parts of the plant are used by populations from many countries for the treatment of several diseases such as leprosy, epistaxis, eye problem, elimination of intestinal worms, anorexia, skin ulcers, biliousness, skin diseases like ringworms, burning sensation, wounds and itching. It is also used as analgesic, alternative and curative of fever and urinary disorder [10]. Hypoglycaemic activity aqueous extract of neem leaves significantly decreases blood sugar level and prevents adrenaline as well as glucose-induced hyperglycaemia. Recently, hypoglycaemic effect was observed with leaf extract and seed oil, in normal as well as alloxan-induced diabetic rabbits [11].

Literature Review:

2.1Antimicrobial Activity:

Various medicinal plants have been used for years in daily life to treat diseases all over the world [12]. Azadirachta indica, known as neem (Meliacea family), has insecticide and pesticide properties, and many

studies have shown their efficacy as antifungal, anti-inflammatory, among others [13].The global scenario is now supporting the development of modern drugs from less toxic plant products with proven medicinal properties. Each part of neem plant (Azadirachta indica) reportedly has various medicinal properties and has been in use in many continents [14].

2.2 Biological Activity:

A. indica is a very useful traditional medicinal plant in the sub-continent and each part of the tree has some medicinal properties. The plant is native to Asia, but has now naturalized in West Africa and is widely cultivated in Nigeria as an ornamental as well as medicinal plant [15]. In traditional literature, preparations of the tree are claimed to be vulnerable in wide spectrum of diseases. Especially for inflammation-related diseases a good correlation is found with the results of recent experimental investigations. In addition, a variety of other biological activities are reported [16]. A total of 70 compounds from an Indian medicinal plant source (Azadirachta indica or Neem) were virtually screened against these two proteins and further analyzed with molecular dynamics simulations, which resulted in the identification of a few common compounds with strong binding to both structural proteins. The compounds bind to biologically critical regions of membrane and envelope, indicating their potential to inhibit the functionality of these components [17]. According to healing system of Ayueda the Neem tree will strengthen an individual resistance to disease, Neem efficiently regulate immune system and other function. Neem is used for a wide range of disorder incorporate diarrhea, inflammatory diseases, antimicrobials, skin diseases, urinary disorders, antiseptics, bronchitis, cough, asthma, fever, hypertension, and infected burns skin diseases, malaria and many more ailments [18]. Neem has become important in the global context today because it offers answers to the major concerns facing mankind. Azadirachta indica is a fast-growing evergreen popular tree [19].

2.3 Anti-Inflammatory Activity:

Azadirachta indica locally known as Neem, is reported to have significant anti-bacterial, immunomodulatory and anti-inflammatory activities, which are complementary to wound healing process [20].

2.4 Antioxidant Activity:

Neem (Azadirachta indica) is a member of the Meliacea family and its role as health-promoting effect is attributed because it is rich source of antioxidant. It has been widely used in Chinese, Ayurvedic, and Unani medicines worldwide especially in Indian Subcontinent in the treatment and prevention of various diseases [21].

2.5 Anticancer Activity:

All parts of A. indica, particularly the leaves, bark, seed-oil and their purified products are widely used for

treatment of cancer [22]. The safety of these compounds is not always taken into consideration and few in vivo toxicity studies have been performed. The current study is a literature review of the latest in vivo toxicity of A. indica. It is divided in two major sections—aquatic animals' toxicity and mammalian toxicity each related to neem's application as a pesticide or a potential new therapeutic drug, respectively [23]. It is now considered as a valuable source of unique natural products for development of medicines against various diseases and also for the development of industrial products [24].

2.6 Therapeutic Use of A. Indica:

Neem tree parts have also been used as a general folk medicine, and more recently, its constituents have been purified and found to possess greater antioxidant, hepatoprotective, antimicrobial, and anticancer activities. Moreover, its constituents also show therapeutic implications in the modulation of cell signaling pathways involved in the management of cancer [25]. Neem is used in various diseases like dermatitis eczema, acne, bacterial and fungal infections and other skin disorders. It is now being used in the manufacture of modern day medicinal, cosmetics, toiletries and pharmaceuticals [26].

2.7 Antisecretory Activity:

Indigenous uses of A. indica (Meliacea) (locally known as neem) leaves for curing gastrointestinal disorder such as diarrhea and cholera is wide spread. The objective of the present study was to evaluate the antibacterial and antisecretory activity of neem extract against Vibrio cholerae, a causative agent of watery diarrhea such as cholera [27].

2.8 Neem Helps Boost Your Immune System:

All the parts of the Neem tree – Leaves, Flowers, Seeds, Fruits, Roots and Bark have been used traditionally for the treatment of eczema, ringworm, acne, inflammation, hyper glycaemia, chronic wound infections, diabetic foot, and gas gangrene, Fever, Skin Diseases and Dental disorders. Neem helps boost your immune system [28]. Neem not only helps in curing diseases, but it also provides us with the strength of fighting diseases by enhancing our immunity [29].

3.1 Materials And Methods

Sample Collection

It ensured that the plant was healthy and free of germs. The fruits were washed under running tap water to remove dust and other external particles as well as to clean the fruits and a certain number of fruits dried in the shade and new fruits were stored.

Solvent Extract: Dried and fresh fruits are crushed into small pieces, powdered and mixed in a ratio of 1:10 with methanol, and distilled water separately. Extraction was obtained by continuous grinding using mud and pestle followed by filtration using filter paper. The filters were then dried in a vacuum using a

rotating evaporator and concentrated stored at 4oC for further studies. The residues were also dispersed with appropriate solvents for antibacterial testing [30].

Test For Alkaloids

Each dry extract powder (100 mg) was dissolved in 5 ml of methanol and then filtered. 5 ml of hydrochloric acid (1%) was mixed with 2 ml of the filtrate, and then 1 ml of the mixture was taken separately in two test tubes. Few drops of Dragendorff's reagent (potassium iodide bismuth nitrate) were added in the tube and appearance of orange precipitate was taken as positive. Few drops of Mayer's reagent (composed of mercuric chloride and potassium iodide dissolved in distilled water) were added to the second tube and appearance of buff-coloured precipitate designates the existence of alkaloids.

Test For Saponins:

Distilled water 10ml was mixed with 1g of powdered dry extract and then boiled and filtered.

The filtrate was mixed again with 3 ml of distilled water and shaken for 5 min.

Appearance of foam after shaking designates the existence of saponins.

Test For Flavonoids:

500 mg of powdered dry extract was added to 5 ml of ethanol, the mixture is slightly heated and then filtered. The filtrate was added to some pieces of magnesium chips, a few drops of concentrated HCl was then added to the mixture. Occurrence of a pink, orange, or red to purple coloration designates the existence of flavonoids.

Test For Tannins:

500 mg of powdered dry extract was added to 10 ml of distilled water, the mixture is then filtered and few drops of 1% ferric chloride solution are added to the filtrate. Occurrence of a blue-black, green or blue-green precipitate designates the existence of tannins [31].

3.2 Antibacterial Activity:

Various decoctions of plants materials, in folk medicine, are used to treat infections. These plant materials are used for the treatment of wounds or for relief from burns in the form of poultice(s) or household remedies. Infectious diseases cause approximately one half of all deaths, especially, in the tropical regions of the world [32]. We performed our screening against different pathogens while keeping in view their clinical significance.

Materials:

Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella typhi, E. coli were used, available at Centre of Biotechnology and Microbiology lab, University of Bacha khan. Organic solvents used in the experiments were of analytical grade. Others materials include nutrient ager, sterile cork borers, petri

dishes (14 cm), micropipettes, incubator, test samples (plant Ext and Aq Ext).

Procedure:

The crude extract and Aq Ext of the plant were screened for possible antibacterial activity against the above-mentioned pathogens. The experiment was performed by following steps as per our reported procedure. [33]

1.Nutrient ager medium was prepared, poured into sterile test tubes and incubate for 24 hours at 37 oC to check its sterility.

2.After incubation for 24 hours, the test organisms were inoculated to the sterile nutrient broth and incubated for 24 hours at 37 oC.

3.Nutrient agar medium was prepared on the same day of the experiment and poured into sterile petri dishes. The plates were incubated overnight for sterility test at 37 oC.

4.After sterility test of the medium, test cultures (18-24 hours old) from the nutrient broth were transferred and evenly distributed over the plates under the laminar air flow.

5.After introducing the test culture to plates, wells (6 mm) were made with a sterile borer.

6.Sterile DMSO (< 1%) was used for preparation of stock solutions (test samples) at a concentration of 3 mg/ml.

7.From the stock solution, 100 µl of the test samples was poured into the respective wells, using a sterile micropipette.

8.Results were recorded after 24 hours of incubation at 37 oC.

9.3.3 Antifungal activity:

Materials:

Test Fungi: Aspergillus Niger, Penicillium notatum, Fusarium oxysporum, Sabouraud Dextrose Agar (SDA), autoclave, incubator, micropipettes, magnetic stirrer, dimethyl sulfoxide (DMSO), screw test tubes, and test samples (Cr. Ext. and Aq Ext).

Procedure:

The anti-fungal activity of the test samples was performed as per our reported procedure [34]. Stock solutions (24 mg/ml) of Cr. Ext and Aq Ext were prepared in DMSO. Sabouraud dextrose agar (SDA) medium, for the growth of fungal species, was prepared as per our reported procedure [35]. Aliquots 4 ml and 66.6 µl from the stock solution were transferred to screw test tubes that were sterilized using moist steam sterilization method. The test tubes were allowed to cool in slanting position. The final concentration became 400 µg/ml of SDA. Fresh culture of each specie was inoculated to test tubes. Other test tubes supplemented with DMSO and standard drugs were used as negative and positive

control, respectively. All the test tubes were incubated for 3-7 days at 27±1°C. On day 7th, the visible non-mycelial linear growths (mm) of the microorganisms were measured and percent linear growth inhibitions of fungal strains were calculated by comparison with reference drug.

3.4 Insecticidal Activity

Material

Test insects (Tribolium castaneum, Rhyzopertha dominica and Callosobruchus analis) organic solvent (methanol), growth chamber, test sample, standard insecticidal drug (Permethrin), petri dishes (9 cm diameter), micropipette (1000 µl), filter paper, glass vials and brush.

Preparation of Test Sample:

Plant's crude extracts 1mg were dissolved in 3 ml 200 of volatile solvent for preparation of the stock solution.

Rearing Technique:

The stored grain pests were reared in plastic bottles containing breeding media (sterile) under controlled conditions required for the rearing of pests. The insects, selected for the experimental work, were of uniform size and age.

Procedure:

The contact toxicity assay [36] was performed as following on first day, the filter paper was cut, equal in size and placed it in petri dishes of about 9 cm or 90 mm in size. Sample solutions of the crude methanolic extracts and Aq Ext were loaded to the petri dishes using a sterile micropipette. The plates were left overnight to evaporate the organic solvents from the samples completely. After complete evaporation of organic solvents from the petri dishes, on second day, 10 healthy insects small in size from each species were selected and transferred with the help of a clean brush to each plate including test samples and control. The plates containing samples and insects were incubated for 24 hours at 27°C in growth chamber with 50% relative humidity.

After incubation, the results were recorded by counting the number of survived insects in each plate.

3.5 Phytotoxic Activity

Materials

The test samples (Cr Ext. and Aq Ext) were tested for phytotoxic bioassay against Lemna minor. Micro-pipettes, flasks, growth chamber, test samples and organic solvent (methanol) were used in the experiments.

Procedure:

The plant extracts were screened for phytotoxic bioassay [37]. The experiment was performed by following steps.

1.Stock solutions (20 mg/ml) of the test samples were prepared in methanol for the growth of L. minor.

2.From the stock solution, test sample at concentration of 10, 100 and 1000 µg/ml were poured to flasks and left at room temperature to evaporate the organic solvent.

3.After evaporation of the organic solvent, 20 ml of the media was added to all flasks.

4.Sixteen healthy L. minor plants were selected, from three fronds and put in respective flasks.

5.The flasks containing L. minor and media were incubated at 27±1oC in growth chamber for seven days. Results were noted after seven days of incubation.

3.6 Hemagglutination Activity

Materials

Materials required for hemagglutination are; all blood group samples, test tubes, centrifuge, incubator and phosphate buffer (pH 7.0).

Procedure:

Hemagglutination activity of test samples was performed according to procedure [38] Phosphate buffer (pH 7.0) was prepared by the ratio of 3:7 (V/V). Stock solution (1 mg/ml of DMSO) was prepared and different dilutions i.e. 1:2, 1:4, 1:8 and 1:16 were made in phosphate buffer, from the stock solution. Fresh blood samples, from healthy persons, were collected on the same day of the experiment and centrifuged, the plasma was discarded and red blood cells were collected for the next steps. 2% RBC's suspension was prepared in the phosphate buffer. From each dilution 1 ml of sample was taken in a test tube and then adds 1 ml of the RBC's suspension to the sample. Incubate the test tubes for 30 minutes at 37oC. After incubation the test tubes were examined for the button formation, indicating negative results.

Chapter:4

Result and Discussion

The findings of the preliminary Phytochemical investigations and the results of antibacterial activity were depicted. The preliminary phytochemical tests performed were of qualitative type and from the phytochemical investigations it was observed that alkaloids, tannins, flavonoids, saponins were present in the extracts.

Table: 4.1 Phytochemical analysis of Neem fruits (Azadirachta indica)

S. No.	TEST	Results	Remarks
1	Alkaloids	+	positive
2	Flavonoids	+	positive
3	Saponins	+	positive

4	Tannins	+	positive

4.1 Antibacterial Activity:

Crude MeOH extract and Aq Ext of A. indica were screened against the test pathogens for possible antibacterial activity. The MeOH presented significant activity against S. aurous (90%), moderate against P. aeruginosa (65%), while low activity E. coli (50%), and S. typhi (60%). The aqueous Ext of the plant showed moderate antibacterial activity against P. aeruginosa (45%) and S. aureus (75%) and showed low activity against E. coli (40%) and S. typhi (35%)

The above results indicated that the MeOH Extract fraction of the plant showed significant activity up to 90% as compared to the standard, which revealed that the plant contains potent antibacterial constituents.

Table 4.2 Antibacterial activities of MeOH Ext and Aq Ext of A. indica fruits against bacterial species.

Name of Bacteria	% Zone of Inhibition	
	MeOH Ext	Aq Ext
E. coli	50%	40%
S. aurous	90%	75%
P. aeruginosa	65%	45%
S. typhi	60%	35%

Fig 4.1 Antibacterial activity of MeOH Ext of A. indica fruits against tested pathogens.

Fig 4.2 Antibacterial activity of Aq Ext of A. indica fruits against bacterial species.

Fig 4.1 SDA media preparation

Fig 4.2 plates prepare for antibacterial activity

Fig 4.3 Antibacterial activity of MeOH Ext

4.2 Antifungal Activity:

The MeOH Ext and its Aq Ext were screened for their antifungal activity. The results indicated that against the A. Niger and F. oxysporum MeOH Ext showed low activity of 10, 5 and 10% respectively. The MeOH Extract and aqueous Ext of the plant were inactive against P. notatum. The remaining test samples were found inactive against, P. notatum. The A. indica results indicate that show low antifungal

agents against P notatum and show good result against A. Niger and F. oxysporum.

Table 4.3 Antifungal activity in MeOH Ext and Aq Ext of A. indica fruits against fungal species.

Fungus species	Percent linear inhibition			
	-ive Control	+ive Control	crude Ext	Aq Ext
F. oxysporum	0	100	20	20
A. Niger	0	100	20	20
P. notatum	0	100	0	0

Fig 4.3 Antifungal activity of crude extract and Aq Ext of A. indica fruits against fungal specie

Fig 4.4 SDA media preparation

Fig 4.5 Slant preparation for antifungal activity

Fig 4.6 Result of antifungal activity in slant

4.3 Insecticidal Activity:

The crude extract and Aq Ext of Tribolium castaneum Callosobruchus analis, and R. dominica were screened for insecticidal activity, using impregnated filter paper method, against three different insect species. Crude extract and Aq Ext of the plant, showed no insecticidal activity against the tested insects. Permethrin was used as positive control while the organic solvent (DMSO) was treated as negative control. The positive control shows 100 % mortality while the mortality rate was 0% in case of negative control. From the results it may be concluded that A. indica fruits shows no insecticidal activity.

Table 4.4 Insecticidal activity of crude extract and Aq extract of A. indica fruits against insecticides.

Insects	Control +ive	Control -ive	Crude Ext	MeOH Ext
T. castaneum	100	0	0	0
R. dominica	100	0	0	0
C. analis	100	0	0	0

Fig 4.7 10 µl in MeOH Ext

Fig 4.8 100 µl in MeOH

Fig 4.9 1000 µl in Ext

Fig 4.10 1000 µl in DMSO solution

4.4 Hemagglutination Activity:

Crude extract and Aq Ext of A. indica fruits at different dilutions were screened for possible hemagglutination activity against human erythrocytes of all blood groups (ABO). The results showed that the MeOH Ext and Aq Ext of the A. indica fruits showed no hemagglutination activity against human RBCs of all blood groups.

Table4.5 Hemagglutination activity of the crude extract and Aq Ext of A. indica fruits against human red

blood cell.

Blood group	1:2	1:4	1:8	1:16
AB-	_	_	_	_
B-	_	_	_	_
A-	_	_	_	_
B+	_	_	_	_
A+	_	_	_	_
AB+	_	_	_	_
O+	_	_	_	_
O-	_	_	_	_

4.5 Phytotoxic Activity:

Herbicides of the A. indica origin are environmentally friendly that necessitates for their search. The phytotoxic activity of crude extract and Aq Ext of A. indica fruits against Lemna minor. The methanolic extract and aqueous Ext growth inhibition at concentrations of 1000 and 100 µg/ml, respectively. While no phytotoxic activity was observed at concentration 10 µg/ml. Paraquat was used as standard plant growth inhibitor.

Table4.6: Phytotoxic activity of crude extract and Aq Ext of A. indica fruits against Lemna minor

Conc (µg/ml)	MeOH Ext	Aq Ext	Control
Lemna Minor	10 µg/ml	11	12	16
100 µg/ml	15	14	16	
1000 µg/ml	16	15	16	

Fig 4.4 Phytotoxic activity of the crude extract and Aq Ext of A. indica fruits against L. minor

4.6 Discussion:

Azadirachta indica has been shown to have significant effects on both gram-positive and gram-negative organisms and other bacteria that cause a wide array of human and animal diseases. Antibacterial activities of A. indica against some bacterial pathogens.

Results obtained during assay with aqueous and methanolic extracts from neem fruits showed their inhibitory effect at all used concentrations against pathogenic fungi included Aspergillus species which

are known to cause aspergilloses.

The crude extract and Aq Ext of A. indica were screened for insecticidal activity, against three different insect species i.e. Tribolium castaneum, Rhyzopertha dominica and Callosobruchus analis. Herbicides of the plants' origin are friendly to the environment that necessitates for their search.

Azadirachta indica is a medicinal plant and is widely distributed around the world. Azadirachta indica commonly known as neem. Indica trees can reach a height of 15–30 m (49–98 feet). In Pakistan, Neem is found in Sindh and in other parts of the southern Punjab. A. indica is useful for fatigue, coughing, fever, loss of appetite, worm infections. It cures cuts and dysfunctional conditions of vomiting, skin diseases, dehydration, and diabetes. The most common infections occur in the mouth, genitals, nails, and skin, hot body parts such as the axillae. Active antiviral effect of neem fruit against Small pox, polio and HSV with a virus inhibition assay. Extracts of bark, leaves, fruits and roots have been used to control leprosy, intestinal helminthiasis and respiratory disorders in children. Neem is useful in treating many ailments, including bacterial infections. A. indica is a rich source of antimicrobial agents.

4.7 Summary:

It is concluded from my research work that the plant materials of A. indica fruits were screened for the presence of natural products i.e. alkaloids, flavonoids, saponin and tannin.

Crude methanol extract and Aq Ext of A. indica fruits were screen for various biological activities including, Antibacterial, Antifungal, Phytotoxic, Hemagglutination, and Insecticidal activity. A. Indica is useful in treating many ailments, including bacterial infections. A. indica is a rich source of antimicrobial agents. The crude extract and Aq Ext of A. indica fruits were screened for biological activity. Herbicides of the plants' origin are friendly to the environment that necessitates for their search. The Biological screening revealed that the plant possesses significant insecticidal, hemagglutination, phytotoxic activity. Moderate antifungal was also observed. The plant was also found to exhibit good antibacterial activity.

Chapter 5:

Conclusions and Recommendations:

The results showed that the aqueous extract of neem fruit was effective as a natural pesticide against wild and insect pests, with comparable or better performance than other common pesticides. A. indica is a medicinal plant and widespread plant known as neem. A. indica plant is useful for fatigue, coughing, fever, loss of appetite, worm infections Most often the infection occurs in the mouth, genitals, nails, and skin, especially in warm, wet areas such as the axillae. A. indica is a rich source of antimicrobial agents. Active antiviral effect of neem fruit against Small pox, polio and HSV with a virus inhibition assay. A. indica is useful in treating a number of diseases, including bacterial infections, fungal diseases, viral

diseases.

Recommendation:

Isolation, purification, and structure elucidation of biological active compound.

In vivo pharmacological evaluation of that isolated compound.

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