International Journal of Research in Chemistry and Environment
Vol. 2 Issue 1 January 2012(33-37)
ISSN 2248-9649

Research Paper

Evaluation of the Antibacterial Potential of *Azadirachta indica* Leaf Extracts

Jahan Mumtaz[1], Warsi M.K.[2],*Khatoon Fehmeeda[1], Hamid Hinna[3]
[1]Department of Applied Science & Humanities, Jamia Millia Islamia (Central University) New Delhi, INDIA
[2]Deparment of Biosciences, Jamia Millia Islamia (Central University) New Delhi, INDIA
[3]Deparment of Chemistry, Jamia Hamdard, New Delhi, INDIA

Available online at: www.ijrce.org

(Received 19th September 2011, Accepted 8th November 2011)

Abstract - *The current investigation was designed to evaluate the antibacterial activity of leaf extracts of Azadirachta indica* (*Meliaceae*) *commonly known as neem against some gram-positive* (*Staphylococcus aureus*) *and gram-negative* (*Escherichia coli and Pseudomonas florescence*) *bacteria by disc diffusion method. Phytochemical studies show the presence of saponins, tannins, glycosides, flavonoids, steroids, coumarins and alkaloids. Ethanol extracts* (*50 mg/mL*) *exhibited the highest activity 12, 20, mm zone of inhibition* [*s. aureus and E. coli*], *Petroleum-ether extracts* (*or control*) *exhibit the least activity.*

Keywords: Antibacterial activity, Disc diffusion method, Extracts MIC, Phytochemicals, Zone of inhibition etc.

Introduction

Natural drugs have been a part of the evolution of human, healthcare for thousands of years. Nowadays nearly 88% of the global populations turn to plant derived medicines as their first line of defense for maintaining health and combacting diseases. One hundred and nineteen secondary plant metabolites derived from plants are used globally as drugs,–15% of all angiosperms have been investigated chemically and of that 74% of pharmacologically active plant derived components were discovered [1]. Plants are rich in a wide variety of secondary metabolites such as tannins terpenoids, alkaloids, flavonoids, etc. which have been found in vitro to have medicinal properties. Pharmacological studies have accepted the value of medicinal plants as potential source of bioactive compounds [2]. Phytochemicals from medicinal plants serve as lead compounds in antimicrobial discovery [3,5].

Literature reports revealed that natural materials are as sources of new antibacterial agents. Different extracts from traditional medicinal plants were tested and some natural products were permitted as new antibacterial drugs. There is still an urgent require to identify novel substances active against pathogens with higher resistance [6,8]. Lot of works reports antibacterial and phytochemical constituents of medicinal plants and their use for the treatment of microbial infections (both topical & systemic applications) as possible alternatives to chemically synthetic drugs to which many infectious microorganisms have become resistant. All through the last few years the pace of development of new antibacterial drugs has slowed down, while the prevalence of resistance (especially multiple) has increased astronomically [9,10]. Literature information and ethno-botanical records recommend that plants are the sleeping giants of pharmaceutical industry[11] and provide natural source of antimicrobial drugs that provides novel compounds that may be engaged in controlling some infections globally.

Azadirachta indica (commonly known as Neem) is most frequently used traditional medicinal plant. Nearly all parts of the plant are endowed with medicinal property. For the period of the last few decades, apart from studies in the chemistry of *Azadirachta indica* compounds, considerable progress has been made in evaluating biological activity of phytochemicals and antibacterial for medicinal applications. In the modern age, the plant is considered as a valuable source of unique natural products for development of medicines against various diseases [3, 12]. Due to traditional medicinal uses of *Azadirachta indica*, this study was conducted to ascertain it's potentially antibacterial and pharmacologically activity.

Material and Methods

Sample Collection and Extraction Procedures

Fresh and matured leaves of *Azadirachta indica* were collected from the Jamia Millia Islamia University campus and washed by sterilized distilled water followed by washing in mercuric chloride solution (0.1%) and again washed in sterilized distilled water.

Ethanol Extract

Azadirachta indica leaves (100 g) were ground into fine powder[13] using a stainless-steel grinder, and deep in 100% ethanol (200 mL) for overnight. The ethanol fraction

was separated using sterile muslencloth and filter through sterile Whatman filter paper (no. 02). The filtered extract was concentrated by a rotary film evaporator.

Aqueous Extract

Plant material (100 g) was crushed in sterile water (250 ml) for preparation of aqueous extract[13]. The extract was separated using sterile muslin cloth and filter through sterile Whatman filter paper (no. 02).

Chloroform, Petroleum ether, Acetone Extract

For preparation of chloroform, petroleum ether and acetone extract, ground plant sample (100 g) was added in chloroform, petroleum ether and acetone respectively (200 ml each case) and left for overnight at room temperature. [15]. The extracts were separated using sterile muslin cloth and filter through sterile Whatman filter paper (no. 02).

Source of microorganisms

The organisms used were *Staphylococcus aureus*, *Escherichia coli and Pseudomonas florescence*. The organisms were obtained from the Microbial Lab of Department of Biosciences, Jamia Millia Islamia, India.

Screening for antibacterial activity

Antibacterial activity of extracts was carried out by filter paper disc diffusion method [16, 17]. For antibacterial activity the microorganisms were cultured in Nutrient Broth at 37° ± 1°C for overnight. First we prepared sterile nutrient agar plates in incubator, placed in it for 24 hrs. After 24 hrs select the nutrient agar plates which were not contaminated. With the help of spreader spread 100 µl inoculums (Bacteria) in solid nutrient agar plates until the agar surface in the plates absorb all the inoculums. Subsequently, paper discs (mention the filter paper no. and diameter of disc) which were sterilized and soaked in different extracts (50 mg/disc) allowed to dry completely and placed on nutrient agar plate. Filter paper discs soaked in sterile distilled water and allowed to dry were used as control. The Petri dishes were incubated at 37° ± 1°C for 24 hrs.

Minimum inhibitory concentration

The minimum inhibitory concentration (MIC) is defined as the lowest concentration of the antimicrobial agent that will inhibit the visible growth of a microorganism after overnight incubation [18- 20]. MIC of *A. indica* extracts were determined by macrodilution broth method [20]. Two ml of different extract solutions at the different concentrations (0.5% w/v to 0.040% w/v) was mixed with 15 mL of sterile molten agar in conical flask. Final concentration of *A. indica* in Nutrient agar was 0.5% w/v to 0.040 % w/v respectively. The mixture was well mixed before being poured into sterile Petri dishes containing 15 ml hard agar. The 02 ml of 40% v/v solvent (ethanol, chloroform, petroleum ether and acetone) without added test materials was used as the negative control. The cultures (05 µl) were taken from nutrient broth and added to three places on the medium surface and incubated at 37°± 1°C.

Phytochemical Screening

Screening was carried out on *A. indica* extracts to determine the active compounds by using the procedures of Sofowora [20-22]. Extract was measured into a test tube for each of the tests and concentrated by evaporating the extractant in a trough. Tests were carried out for carbohydrates, reducing sugars, tannins, polyphenols, lipids, flavonoids, ketones, alkaloids, steroids resins, tannins, saponins, natural rubber, gums, waxes, dyes, pharmaceuticals and many specialty products [23, 24] and triterpenes. The screening was carried out in the department of Chemistry of the Jamia Millia Islamia, India.

Results and Discussion

The phytoconstituent of *A. indica* extract showed alkaloids, saponins steroid, flavonoids, glycosides and tennins respectively as shown in (Table 02). Various scientists isolated 140 compounds from different parts of neem[25]. Literature report revealed that glycosides, flavonoids. alkaloids, tannins, steroids were present in different parts of *A. indica*. The bioactive products of *A. indica* have been used in treatment of various ailments since time immemorial, role of phytochemical in inhibition of growth of microorganisms has gained less prominence [26-29]. In the present study, petroleum ether, chloroform, ethanol, acetone and aqueous extract of leaves of *A. indica* were tested against selected gram-positive and gram-negative bacterial strains [Table 01(a,b)]. The leaf extracts exhibited the growth of tested gram-positive and gram-negative bacterial strains. Crude glycosides, flavonoids, coumarins, tannins, steroids, alkaloids and saponins are responsible for inhibition of bacterial growth. Among the different extracts, ethanolic leaf extracts of *A. indica* was found to be more active towards the bacterial strains used in the study.

Conclusion

The results of this work show that the *A Indica* possesses antibacterial properties, which can be used as natural antimicrobial agents for human and infectious diseases preservation. Moreover, the development of natural antimicrobial agents will help to decrease negative effects (pollution in environment, resistance) of synthetic chemicals and drugs.

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Table 1(a): Antibacterial screening of A. *indica* leaf extract using disc diffusion method.

Test Organisms	*A. indica* extract/mean presence of antibacterial activity				
	Petroleum ether	**Acetone**	**Ethanol**	**Aqueous**	**chloroform**
Control	-	-	-	-	-
S. aureus	+	+	+	+	+
E. coli	-	+	+	+	-
P. florescence	+	-	+	-	-

Zone values are means ± S.D ; +: *Represent an inhibitory effect;_*: *Represent no inhibitory effect*
(Paper dish used as control)

Table 1(b): Antibacterial screening of A. *indica* leaf extract using disc diffusion method.

Test Organisms	Zone of inhibition in mm				
	Petroleum ether	**Acetone**	**Ethanol**	**Aqueous**	**chloroform**
Control	-	-	-	-	-
S. aureus	9	13	12	13	14
E. coli	-	12	20	12	-
P. florescence	7	-	8	-	-

Zone values are means ± S.D

+: *Represent an inhibitory effect*

_: *Represent no inhibitory effect*
(Paper dish used as control)

Table 2: Phytochemical analysis of leaf extracts of *A. indica*

S. No.	Phytochemical	Result	Part of *A. indica*	Test
1.	Terpenoid	+	Fruits, Leaves, Bark, Seed Oil, Trunk Bark, Twigs	Lieberman-Buchard, Un-saturation test due to presence of double bond.
2.	Steroids	+	Leaves, Flowers, Bark	Libermann Bruchard
3.	Flavonoids	+	Leaves, Bark, Flowers	Vanillin HCl, Pew's
4.	Coumarin	+	Leaves, Bark, Twigs, Fruits	Fluorescence
5.	Hydrocarbons	+	Leaves, Flowers	Bromine
6.	Alkaloids	+	Leaves, Bark, Twigs, Fruits	Dragendorff's
7.	Combined anthraquinone	_	_	Borntrager's+ Sulphuric acid
8.	Monosaccharide	_	_	Barfoed's test
9.	Anthraquinone derivatives	_	_	Borntrager's
10.	Amino acid	+	Seed oil	Ninhydrin test
11.	Carbohydrates	+	Seed oil ,Leaves, Flowers	Molish
12.	Glycosides	+	Leaves, Flowers	Haemolysis
13.	Redusing Sugar	+	Leaves	Fehling's
14.	Pentoses	+	Leaves	Salkowski's, Standard
15.	Tannins	+	Leaves	Ferric chloride
16.	Saponins	+	Leaves	Frothing

indicate the presence of the constituent
- *indicate the absence of the constituent*

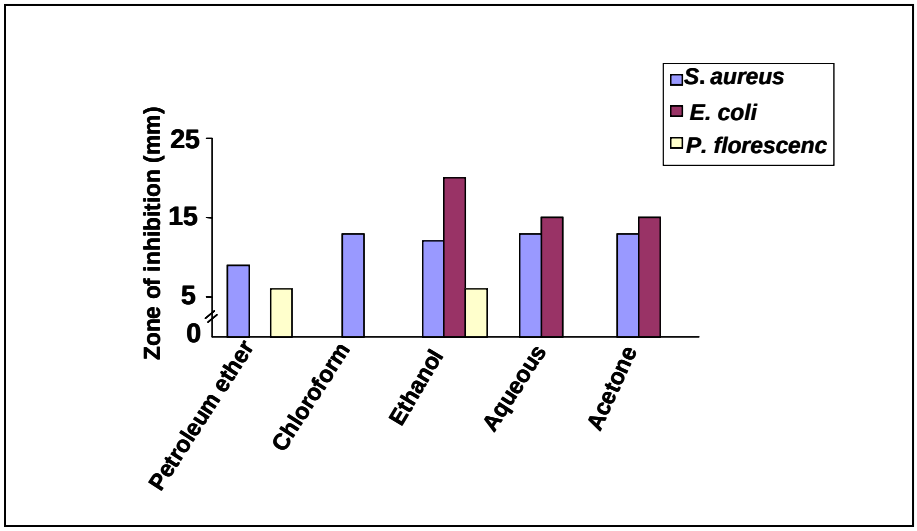

Figure: 1 Activity of leaf extract of *Azadirachta indica* in different solvents

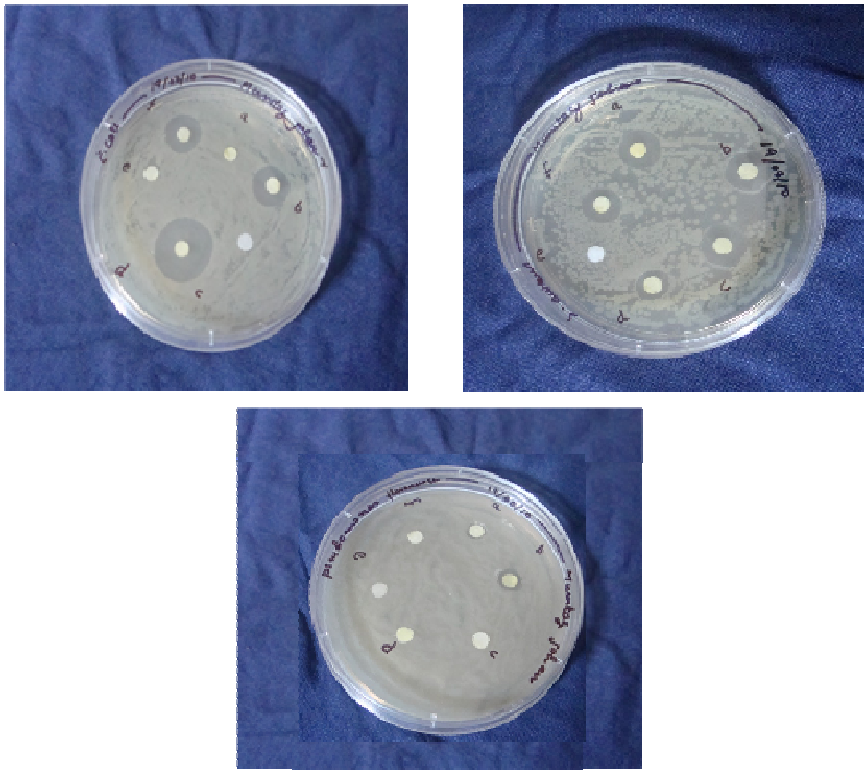

Figure 2: Zone of Inhibition against -
(A) *E. coli*. a- control, b- aqueous, c- petroleum ether, d- ethanol, e- chloroform, f- acetone. (Paper dish used as control)
(B) *S. aureus* a- acetone, b- aqueous, c- chloroform, d- petroleum ether, e- control, f- ethanol. (Paper dish used as control)
(C) *P. florescence* a- petroleum ether, b- ethanol, c- chloroform, d- acetone, e- aqueous, f - control. (Paper dish used as control)