



King Saud University

Saudi Journal of Biological Sciences

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ORIGINAL ARTICLE

Analysis of anti-bacterial and anti oxidative activity of *Azadirachta indica* bark using various solvents extracts



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Received 9 February 2015; revised 24 July 2015; accepted 3 August 2015

Available online 6 August 2015

KEYWORDS

Anti-bacterial activity;
Anti-oxidant activity;
Azadirachta indica

Abstract Herbal medications have been used for relief of symptoms of disease. Regardless of the great advances observed in current medicine in recent decades, plants still make a significant contribution to health care. An alarming increase in bacterial strains resistant to a number of antimicrobial agents demands that a renewed effort be made to seek antibacterial agents effective against pathogenic bacteria resistant to or less sensitive to current antibiotics. Anti-bacterial activity of *Azadirachta indica* stem bark was tested against pathogenic *Salmonella paratyphi* and *Salmonella typhi* using various solvent extracts. The in vitro anti-bacterial activity was performed by agar well diffusion method and the results were expressed as the average diameter of zone of inhibition of bacterial growth around the well. The ethanol and methanol extracts showed better anti-bacterial activity with zone of inhibition (20–25 mm) when compared with other tested extracts and standard antibiotic Erythromycin (15 mcg) with zone of inhibition (13–14 mm). Using Fisher's exact test of significance difference was found between two *Salmonella* strains sensitivity patterns against tested extracts ($P \leq 0.035$). Extracts of *A. indica* stem bark also exhibited significant antioxidant activity, thus establishing the extracts as an antioxidant. The results obtained in this study give some scientific support to the *A. indica* stem bark for further investigation of compounds and in future could be used as drug.

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1. Introduction

Herbal medications have been used for relief of symptoms of disease (Maqsood et al., 2010). Regardless of the great advances observed in current medicine in recent decades, plants still make a significant contribution to health care. Much interest, in medicinal plants however, emanates from

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Peer review under responsibility of King Saud University.



their long use in folk medicines as well as their prophylactic properties, especially in developing countries (Naima et al., 2012). Natural antioxidants either in the form of raw extracts or their chemical constituents are very effective to prevent the destructive processes caused by oxidative stress (Nandita et al., 2014; Zengin et al., 2011). Although the toxicity profile of most medicinal plants has not been thoroughly evaluated, it is generally accepted that medicines derived from plant products are safer than their synthetic counter parts (Vongtau et al., 2005).

Salmonella is a primary cause of food poisoning worldwide. The Centre for Disease Control and Prevention estimated that approximately 1.4 million cases of salmonellosis were annually reported in the United States (Mead et al., 1999). Certain pathogenic Salmonella serotypes adapted to humans, such as *Salmonella typhi* and *Salmonella paratyphi* usually cause severe diseases in humans, such as enteric fever. Enteric fever (Typhoid) is a global bacterial infection with an annual infection rate of 21.6 million and 10% fatality rate (John et al., 2003). In developing countries, typhoid is more severe due to poor hygiene, indiscriminate use of antibiotics, and a rapid rise in multidrug resistance. Resistance to the first line drugs, chloramphenicol, ciprofloxacin, and amoxicillin, in the course of salmonellosis management has been reported (Zulfigar et al., 1994; Benoit et al., 2003.). Resistance to antimicrobial agents such as antibiotics is emerging in a wide variety of organisms and multi drug resistant organisms pose a serious threat to the treatment of infectious diseases. Hence, plant derived antimicrobials have received considerable attention in recent years.

Azadirachta indica is a versatile tree of family Meliaceae, popularly known as 'Yavan Priya' meaning the beloved of Muslims. Bark has for long been used in the traditional system of medicine for its beneficial properties. The aqueous extract of stem bark is used as tonic, stimulant and as a remedy against various skin ailments (Dhawan and Ratnaik, 1993). It is a multi functional as well as multi utility natural product and without any side effects. The bark contains 3.43% protein, 0.68% alkaloids and 4.16% minerals. Studies showed anti-pyretic, anti-inflammatory (OKpanyi and Ezeukwa, 1981) diuretic (Binde et al., 1958) antiseptic, antibacterial and anti-tumor and interferon inducing activities (Fuziwara et al., 1982).

The main objective of this present study is to evaluate anti-bacterial and anti-oxidative properties of *A. indica* using various extracts.

2. Methodology

2.1. Collection of plant material

The bark of *A. indica* A. Juss (Meliaceae) was collected from local areas (Hyderabad, Andhra Pradesh, India). The Bark was thoroughly washed with distilled water to remove dirt. It was shade dried and ground into a fine powder.

2.2. Preparation of extracts

The material was extracted with six solvents, independently viz. Methanol, ethanol, acetone and aqueous extracts. Briefly, 100 g of the powder was soaked into respective solvent for three days and followed by filtration of the solvent using

Whatman's filter paper under aseptic conditions. A stock solution of the extracts was prepared at the concentration of 200 mg/ml and stored at 2 °C till further use.

2.3. Bacterial strains

Two pathogenic Salmonella species – *S. typhi* and *S. paratyphi* were sub cultured on nutrient agar for every 15 days and maintained on nutrient agar slants at 4 °C. Fresh inoculums were taken for the test.

2.4. Evaluation of anti-Salmonella activity

Anti-bacterial activity of the extract was determined by agar diffusion assay (Reeves, 1989). Salmonella strains were first grown in Mueller Hinton broth (MHB) under shaking condition for 4 h at 37 °C and after the incubation period 0.1 ml of the test organisms inoculum was spread evenly with a sterile glass spreader on Mueller Hinton Agar (MHA) plates. The seeded plates were allowed to dry in the incubator at 37 °C, Wells were made using sterile 6 mm cork borer in the inoculated MHA plate. The wells were filled with 200 µl of the extracts (re-suspended in respective solvents) and negative controls (1:1 [solvent:water]). The concentration of stock extracts was 200 mg/ml. The inoculated plates were incubated at 37 °C for 24 h. The plates were observed for the presence of inhibition of bacterial growth that was indicated by a clear zone around the wells. The size of the zone of inhibition was measured and the anti-Salmonella activity was expressed in terms of average diameter of the zone of inhibition in millimetres. The results were compared with the standard antibiotics, Gentamycin (10 mcg), Ciprofloxacin (30 mg), Erythromycin (15 mcg) and Chloramphenicol (30 mcg). The photograph was taken in UV-Visible documentation system.

2.5. Statistical analysis

Statistical analysis was done using SAS 9.0 version. Salmonella strains which were sensitive to different extracts and various standard antibiotics were compared using Fisher's exact test with $P \leq 0.035$ being considered as significant. Difference in sensitivity pattern of tested extracts was found individually on two Salmonella strains performing *t*-test [*S. paratyphi* $P \leq 0.0004$, *S. typhi* $P \leq 0.0014$], (Table 1) which was considered as significant.

3. Results

A. indica ethanol and methanol extracts showed better zone of inhibition (20–25 mm) when compared with the other tested extracts and standard antibiotic Erythromycin (15 mcg) which showed a lesser zone of inhibition (13–14 mm).

S. typhi showed better sensitivity against methanol extract with zone of inhibition (25 mm) compared with the standard antibiotics Gentamycin and Erythromycin with zone of inhibition (21 and 13 mm). There exists a significant difference among the various tested extracts anti-bacterial activity (*t*-test: $P \leq 0.0014$, $P \leq 0.0004$, Table 1).

Results of total phenolic content of different extracts of *A. indica* stem bark were significant and shown in (Table 3). The

Table 1 Antibacterial activity of different extracts of *Azadirachta indica* stem bark against *Salmonella* strains.

Extracts	Diameter of inhibition zone (mm)	
	<i>S. paratyphi</i> A	<i>S. typhi</i>
Aqueous	10 ± 0.2	17 ± 0.6
Methanol	12 ± 0.4	25 ± 0.3
Acetone	10 ± 0.5	24 ± 0.2
Ethanol	20 ± 0.6	23 ± 0.4
Ciprofloxacin (30 mcg)	31 ± 0.2	30 ± 0.3
Gentamycin (10 mcg)	25 ± 0.1	21 ± 0.2
Chloramphenicol (30 mcg)	30 ± 0.5	27 ± 0.4
Erythromycin (15 mcg)	14 ± 0.3	13 ± 0.5
<i>Negative control:</i>		
Methanol:water (1:1)	0	0
Ethanol:water (1:1)	0	0
Acetone:water (1:1)	0	0
<i>t</i> -Test	<i>P</i> < 0.0004	<i>P</i> < 0.0014
Fisher's exact test	<i>P</i> < 0.035	

Table 2 IC₅₀ value of *Azadirachta indica*.

Extracts	Lipid peroxidation	DPPH
Aqueous	0.708 ± 0.01	0.829 ± 0.021
Methanol	0.370 ± 0.13	0.590 ± 0.0081
Acetone	0.907 ± 0.001	1.494 ± 0.005
Ethanol	0.28 ± 0.01	0.202 ± 0.04
Standard	0.0452 ± 0.3	0.0035 ± 0.2

Table 3 Total polyphenols, flavonoids and antioxidant activity of *Azadirachta indica*.

Extract	Polyphenol ^a	Flavonoids ^b	Total antioxidant activity ^c
Aqueous	0.762 ± 0.29	6.22 ± 0.11	1.48 ± 0.175
Methanol	2.680 ± 0.08	1.14 ± 0.18	3.58 ± 0.08
Acetone	0.592 ± 0.06	0.92 ± 0.02	1.79 ± 0.21
Ethanol	1.462 ± 0.017	0.46 ± 0.08	2.53 ± 0.106

^a Gallic acid.^b Quercetin.^c Ascorbic acid equivalents mg/g dw plant material respectively; results represented in mean ± standard deviation.

total phenol content expressed as gallic acid equivalent (GAE) ethanol and methanol extracts had high polyphenol content compared to aqueous and acetone extracts. Total flavonoid content (Table 3) of different extracts expressed as quercetin equivalents. The aqueous extract had the highest flavonoids content of 6.22 mg QE/ml. Results of the antioxidant measurements are summarized in (Table 3). The highest value was observed in methanol and ethanol extracts.

The radical scavenging effects of the extracts were tested using methanolic solution of the DPPH free radical which exhibits a deep purple color with maximum absorption at 527 nm. In this study, all the extracts showed a dose–response

curve of DPPH free radical scavenging activity, as indicated by the concentration dependent increase in percentage inhibition. Results revealed that ethanol extracts had the higher DPPH radical scavenging and Lipid peroxidation ability than those of the other extracts. IC₅₀ values of DPPH radical scavenging and Lipid peroxidation are presented in Table 2.

4. Discussion

Numerous surveys conducted in different countries of the world, including United States, demonstrated the presence of active compounds in medicinal plants (Srivastava et al., 1996). More than 80 % of the world's population uses traditional herbal medicine for their primary health care (Vijayan et al., 2007). Various plant parts continue to serve as sources of new drugs and chemicals (Tijani et al., 2008). In recent times there has been a marked shift towards herbal cures because of the pronounced cumulative and irreversible reactions of modern drugs. Due to over population, urbanization and continuous exploitation of these herbal reserves, the natural resources along with their related traditional knowledge are depleting day by day (Pande et al., 2007). In the present era of drug development and discovery of newer drug molecules many plant products are evaluated on the basis of their traditional uses. Salmonellosis and enteric fever are always a public health concern in most developing countries, which are mostly low or middle-income countries with inadequate sanitation and hygiene, particularly regarding food, water and disposal of human excreta. In our previous reports we found significant antibacterial activity with crude protein extracts from seeds of six different medical plants against standard bacterial strains (Raid et al., 2014).

An alarming increase in bacterial strains resistant to a number of antimicrobial agents demands that a renewed effort be made to seek antibacterial agents effective against pathogenic bacteria resistant to or less sensitive to current antibiotics (Salman et al., 2008). Many plants have been investigated scientifically for antimicrobial activity and a large number of plant products have been shown to inhibit growth of pathogenic bacteria. A large number of these agents appear to have structures and modes of action that are distinct from those of the antibiotic in current use (Mollazadeh et al., 2010). In our study ethanol and methanol extracts of the *A. indica* were found to show better sensitivity compared with standard antibiotic Erythromycin. Extracts of *A. indica* stem bark also exhibited significant antioxidant activity, thus establishing the extracts as an antioxidant.

5. Conclusion

The results obtained in this study give some scientific support to the *A. indica* stem bark for further investigation of compounds and in future could be used as drug.

Acknowledgements

We are grateful to the Department of Laboratory Sciences, College of Applied Medical sciences, King Saud University for encouragement and providing facilities for carrying out the present study.

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