Antibacterial Activity of *Azadirachta indica* (Neem) Leaf Extract against Bacterial Pathogens in Sudan

Hala A. Mohammed and Al Fadhil A. Omer

Faculty of Medical Laboratory Sciences, Al Neelain University, Khartoum, Sudan

Abstract

Background: *Azadirachta indica* (Neem) is a multipurpose tree with multiple health benefits. Different parts of the tree were shown to exhibit antimicrobial effects against a wide variety of microorganisms. Screening of this medicinal plant for bioactive compounds may lead to development of less expensive new antimicrobial agents with improved safety and efficacy.

Objective: To assess the antimicrobial activity of *Azadirachta indica* (Neem) leaf extractagainst human pathogenic bacterial pathogens;andto compare that with the antimicrobial activity of synthetic antibiotics.

Material and methods: 100 bacterial test strains: *Escherichia coli,Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumoniae, Staphylococcus aureus and Enterococcus faecalis*were enrolled in the study.Ethanolic extracts of *Azadirachta indica*leaves were prepared at varying concentrations and soaked on Whatmann filter paper discs, which were applied on inoculated plates of Muller Hinton agar.Standardized discs ofthe synthetic antibiotics: ciprofloxacin, erythromycin, norfloxacin, co-trimoxazole, ceftriaxone, and gentamicin were also applied on inoculated plates of Muller Hinton agar. The disc diffusion method was used to screen the antibacterial activity of both *Azadirachta indica* leaf extractand synthetic antibiotics.

Results: *Azadirachta indica* leaf extract showed strong antimicrobial activity against all bacterial species studied at all the concentrations tested. It showed maximum inhibition against *Proteusmirabilis* at 6.25mg/ml concentration, when compared with erythromycin ($p = 0.007$). Against *Enterococcus faecalis*, there was a significant difference in the antibacterial activity of the leaf extract at a concentration of 12.5mg/mland those of ciprofloxacin, erythromycin, ceftriaxone, and gentamycin ($p = 0.004$, 0.002, 0.003, and 0.008respectively). **Conclusion:L**eaf extract of *A. indica* (Neem)had exhibited a potent antibacterial activity against various strains of bacterial pathogens.

Keywords: *Azadirachta indica*; antibiotics; pathogenic bacteria; disc diffusion method.

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Introduction

Azadirachta indica (A. indica) belongs to the botanic family *Meliaceae*, commonly known as Neem. It is used in traditional medicine as a source of many therapeutic agents. *A. indica* (leaf, bark and seeds) are known to contain antibacterial and antifungal activities against different pathogenic microorganisms; in addition to antiviral activity against vaccinia, chikungunya, measles, and Coxsackie B viruses[1].

Different parts of Neem (leaf, bark and seeds) have been shown to exhibit wide pharmacological activities such as antioxidant, antimalarial, antimutagenic, anticarcinogenic, anti-inflammatory, antihyperglycaemic, antiulcer, and anti-diabetic properties[2]. The biological activities are attributed to the presence of many bioactive compounds in its different parts.Aqueous extract of Neem leaf extract has a goodtherapeutic potential as an antihyperglycaemic agent in insulin-dependent and non-insulin-dependent diabetes mellitus[3].

Furthermore, Neem leaves may be used for the treatment of various diseases including eczema, ringworm, acne, inflammation, hyperglycaemia, chronic wound infections, diabetic foot, and gas gangrene. They may also remove toxins from the body, neutralize the free radicals present in body, and purify blood. Recently they were reported to act as anticancer agents; and they were shown to have hepato-renal protective activity and hypolipidemic effects[4].

Hence the purpose of our study is to investigate the antimicrobial activity of Neem leaves against human pathogenic bacteria.

Materials and methods

Fresh leaves of Neem (*A. indica*) were collected locally and were air dried in shade. The *A. indica*leaf extractwas then prepared bygrounding 50 g of leaves using mortar and pestle, and the yield was successively soaked by 80 % ethanol for about 72 hours, with daily filtration and evaporation. Solvents were evaporated under reduced pressure to dryness using rotary evaporator apparatus. Filtration and extraction were carried out in the Center of Medicinal and Aromatic Plants,Khartoum (Sudan). Extracts were exposed to air till complete dryness.

The bacterial test strains used were 100 bacterial pathogens, isolated from various clinical specimens: urine, blood,sputum, and wound infections. The clinical specimens were collected for microbiological testing at Soba University Hospital (Khartoum). Bacterial identification was carried out by conventional biochemical methods according to the standard microbiological techniques. These bacterial test strains used were *Escherichia coli*(21),*Pseudomonas aeruginosa* (12), *Proteus mirabilis* (21), *Klebsiella pneumoniae*(21), *Staphylococcus aureus*(17) *and Enterococcus faecalis*(8).

The antimicrobial sensitivity testing was conducted by the agar disc diffusion method. The sensitivity medium (Muller-Hinton agar) was prepared by adding 3.8g of Muller-Hinton agar powder to 100 ml distilled water and autoclaved at 121°C for 15 minutes at 15 lbs., and poured

in sterile Petri plates up to a uniform thickness of approximately 4mm and the agar was allowed to set at ambient temperature before use.The bacterial isolates were suspended in peptone broth and incubated at 37° C for 3-4 hours before used as inocula.The turbidity of the broth culture was adjusted to 0.5 McFarland units. This gives a suspension containing approximately 1-2 x 10^6 colony forming units (CFU)/ml. A sterile cotton swab was inserted into the bacterial suspension, rotated, and then compressed against the wall of the test tube to express any excess fluid. The swab was then streaked on the surface of the Muller-Hinton agar plate. To ensure a uniform, confluent growth, the swab was streaked three times over the entire plate surface.

To test antibacterial activity of Neem leaf extract, it was first dissolved in a methanol solvent, and thenvarying concentrations of the extracts (100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, and6.25mg/ml) were soaked onautoclaved discs of Whatmann filter paper. These filter paper discs were placed on a streaked Muller-Hinton agar plate surface. The plates were incubated overnight at 37° C for 18-24 hours. The antimicrobial activity was detected by measuring zonesof inhibition.To test antibacterial activity of the synthetic antibiotics, standardized discs of ciprofloxacin (5µg),erythromycin (15µg), norfloxacin(10µg), co-trimoxazole(25µg), ceftriaxone (30µg), and gentamycin (10µg) were tested by the agar disc diffusion method by placing on a streaked Muller-Hinton agar plate surface. The antimicrobial activity was also detected by measuring zones of inhibition.

Results

Table I exhibits the antibacterial activity of leaf extract against all tested bacteria at all concentrations. As regard the lowest concentration (6.25 mg/ml) of the leaf extract, its highest antibacterialactivity was detected against *Pseudomonasaeruginosa*(10.6 mm inhibition zone); and its lowest antibacterialactivity was detected against *Escherichia coli*(7.5 mm inhibition zone).

Table I. Mean zones of inhibition (in mm) for differentconcentrations of *A. indica* leaf extract

Bacterial test strains (No. tested)	Concentrations of leaf extract (in mg/ml)				
	100	***50***	***25***	***12.5***	***6.25***
Escherichia coli(21)	14	13	11.8	8.5	7.5
Klebsiella pneumoniae (21)	14.5	12	11.9	8.6	7.8
Proteus mirabilis (21)	14.9	12.8	12.5	11	8.4
Staphylococcus aureus(17)	15	13.7	12.6	11.5	8.8
Pseudomonasaeruginosa (12)	15.8	13.8	12	11.4	10.6
Enterococcus faecalis(8)	15.5	12.5	13.8	11.6	9

Table II exhibits the mean zones of inhibition (in mm) for the different synthetic antibiotics used. Regarding the antibacterial activity of the antibiotics tested, the highest activity was due to the

action of ciprofloxacin against *Pseudomonasaeruginosa*(28 mm inhibition zone); and the lowest activity was due to the action of erythromycin against *Proteus mirabilis*(4.4 mm inhibition zone).

Table II. Mean zones of inhibition (in mm) for different antibiotics

Bacterial teststrains (No. tested)	Antibiotics concentration in (µg/disc)					
	CIP	E	NOR	CO	CEF	G
Escherichia coli(21)	16.9	7.5	9.4	10.7	12	13
Klebsiella pneumoniae (21)	15.8	10	9	6	6.8	12
Proteus mirabilis (21)	27	4.4	19	13	21	12
Staphylococcus aureus(17)	21.7	12.4	14	13.4	13	10
Pseudomonasaeruginosa (12)	28	8.6	18.9	14	15.5	10
Enterococcus faecalis(8)	16.7	11.7	10	9	13.8	10

CIP = Ciprofloxacin(5 µg)CO = Cotrimoxazole(25 µg)E = Erythromycin (15 µg)CEF = Ceftriaxone (30µg)NOR = Norfloxacin(10 µg)G = Gentamycin (10 µg)

There was an insignificant difference ($p > 0.05$) between the antibacterial activity of the leaf extract and the synthetic antibiotics against *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*,and *Staphylococcus aureus*. However, there was a significant difference between the antibacterial activity of the leaf extract and that of erythromycin against *Proteus mirabilis*($p = 0.007$).

Against *Enterococcus faecalis*, there was a significant difference in the antibacterial activity of the leaf extract at a concentration of 12.5mg/mland those of ciprofloxacin, erythromycin, ceftriaxone, and gentamycin ($p = 0.004$, 0.002, 0.003, and 0.008respectively).

Discussion

Many of the existing synthetic drugs cause various side effects. Hence, development of plant-based compounds is required to meetthis demand for production of newer drugs with minimal side effects. *A.indica* leaves possess a good antibacterial activity confirming the great potential of bioactive compounds and is useful for rationalizing the use of this plant in primary health care.

In this study, we have shown that ethanolic extracts of *A. indica*(Neem) leaf to exhibithigh antibacterial activity against all tested bacterial strains at all concentrations used. Several studies had been performed to investigate the antimicrobial activity ofNeem leaf extract and their results were almost similar to our results. One of these studies is the report of Okemo *et al*[5]who stated

that crude extract of Neem plant was very effective against *Staphylococcus aureus*and *E.coli.* They found that an extract concentration of 0.5 mg/ml had significantly reduced *Staphylococcus aureus*inoculum after 24hrs, while extracts with increasing concentrations completely wiped out viable bacteria in a lesser time.

Also Awasthy and his colleagues [6] reported that the ethanol extract of Neem is very useful orally to treat many diseases caused by bacteria.Subapriya and Nagini [7]reported that presence of high concentrations of azadirachtins, quercetin and β-sitosterol in *A. indica* leaves might be responsible for strong antibacterial and antifungal activity. Furthermore, Maragathavalli and his co-authors [8] studied the antimicrobial activities of ethanolic extracts of Neem leaves in various concentration against pathogenic bacteria and comparedthat to gentamycin. They found that the ethanol extract showed maximum inhibition on *Bacillus pumillus, Pseudomonas aeruginosa and Staphylococcus aureus* in an ascending order.

On the other hand, Aslam and her co-workers [9]were able to check the action of Neem extract on three bacterial strains: *Staphylococcus aureus, Corynebacterium bovi* and *E.coli;*and they found a 75 mg/ml concentration was very effective.Also Raja and his colleagues [10] compared the antimicrobial efficacy of aqueous extracts of leafof *A. indica* against human pathogenic bacteria (S*taphylococcus aureus, Enterococcus faecalis, Proteus mirabilis* and *Pseudomonas aeruginosa*). They found that leaf extract exhibited strong antimicrobial activity against these bacteria at all the concentrations tested (500, 1000 and 2000µg/ml).

Conclusion: This study showed that leaf extract of *A. indica* (Neem)has a potent antibacterial activity against various strains of bacterial pathogens.It is recommended to isolate andseparate the bioactive compounds responsible for this antibacterial activityusing advanced scientific techniques.

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