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Research Article



# Antityphoid Activity and Phytochemical Screening of *Azadirachta Indica* Leaf Extracts

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## Abstract

Herbal medicines have been known to human for centuries and practitioners of traditional medicine have described therapeutic efficacy of many indigenous plants for several disorders. The study was aimed to determine the anti-typhoid activity of *Azadirachta indica* leaf extracts against *Salmonella typhi* and *Salmonella paratyphi* isolated from typhoid fever patients. A clinical isolates of *S.typhi* and *S. paratyphi* were obtained from patients attending Murtala Muhammad Specialist Hospital, Kanowere tested against aqueous and methanol extracts of *A. Indica* leavesusing agar well diffusion method. The phytochemical screening of the extract was conducted using conventional laboratory methods. The result showed that the leaf extract of *A. Indica* contain alkaloids, tannin, anthraquinone, flavonoids, phenols and steroids. The result of antibacterial activity of the extracts against the test isolated indicated that the extracts were active against the isolate with higher activity shown by methanol extract (16.0 mm) when compared to aqueous extract (13.5 mm). *Salmonella typhi* was found to be the most sensitive isolate (15.8 mm) than *S. paratyphi* (13.3 mm). It is concluded that the leaf extracts of *A. indica* were active against bacterial isolates associated with typhoid fever.

**Keywords:** Antibacterial activity, *Azadirachta indica*, phytochemicals, *Salmonella typhi*.

## Introduction

Herbal medicines have been known to human for centuries. Practitioners of traditional medicine have described therapeutic efficacy of many indigenous plants for several disorders. This is due to the fact that plants contain many biologically active compounds which have potential for development as medicinal agents [1]. Herbal medicines already form the basis of therapeutic use in the developing countries, but of recent, there has been an increase in the use of herbal medicines in the developed world too [2]. It is likely that plants will continue to be a valuable source of new molecules which may, after possible chemical manipulation and provide new and improved drugs [3]. Bacterial resistance to antibiotics represents a serious problem for clinicians and the pharmaceutical industry and great efforts are being made to reverse this trend, and one of them is the widespread screening of medicinal plants from the traditional system of medicine hoping to get some newer, safer, and more effective agents that can be used to fight infectious diseases [4].

Enteric fever is a systemic bacterial infection caused by the Gram-negative *Salmonella enterica* serovar typhi (*S. typhi*) and the paratyphi serovars A, B and C (*S. paratyphi* A, B and C) of which *S. paratyphi* A is most common [5]. Enteric fever is a generic term for infections caused by both *S. typhi* and *S. paratyphi*. Typhoid and paratyphoid fever refers to the infections caused by the individual serovars [6]. Throughout this work enteric fever will be mostly used, but in cases focusing on *S. typhi* infections typhoid fever will also be used. *S. typhi* has historically been the most common cause of enteric fever but recently there have been several reports on the emergence of enteric fever caused by *S. paratyphi* A especially in Asia [7,8].

Neem plants (*Azadirachta indica)* are mostly trees and rarely shrubs that belong to family Maliacea [9]. It is naturalized in most tropical and subtropical countries. It is broad-leaved evergreen that grows up to 30 m tall. The plant has been used for a long time in agriculture and medicine [4]. Neem is the most versatile, multifarious trees of tropics, with immense potential. All parts of the neem tree-leaves, flowers, seeds, fruits, roots and bark have been used traditionally for the treatment of inflammation, infections, fever, skin diseases and dental disorders [10]. The

medicinal utilities have been described especially for neem leaf. Neem leaf and its constituents have been demonstrated to exhibit immunomodulatory, anti-inflammatory, anti-hyperglycaemic, antiulcer, antimalarial, antifungal, antibacterial, antiviral, antioxidant, antimutagenic and anticarcinogenic properties [11].

In 2012, the antimicrobial activity of leaf extract of neem was conducted against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella typhi* and *Bacillus pumillas* and concluded that ethanol and methanol extract show maximum inhibition on *Bacillus pumillas*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* in ascending order [12]. Neem leaf contains several valuable components such asisoprenoids that include terpenoids containing limonoids, azadirone and its derivatives [13]. The medicinal properties of the plant were studied by several workers, the antimalaria effect [14] antidiabetic effect [15] and anti-fertility effect [16], effect on the central nervous system[17], cardiovascular effect [18] and wound healing [19]. *A indica* has been shown to possess anti-microbial properties by several studies. Rao et al. [20] reported the anti-microbial activity of the seed oil against a variety of pathogens. Oils from the leaves, seeds and bark possess antibacterial action against certain bacteria [21]. Extracts of neem leaf, neem oil and seed kernels are effective against certain human fungi [11]. The study was aimed to determine the antityphoid activity of *A. indica* leaf extracts against *S.typhi* and *S. paratyphi* isolated from typhoid fever patients.

## Materials and Methods

### Ethical approval

Ethical approval (with reference no. HMB/GEN/492/VOL.1) for this research was obtained from the Hospital Service Management Board (HSMB), Kano based on the consent of Murtala Muhammad Specialist Hospital ethical committees

### Test isolates

Two (2) bacterial strains of *S. typhi* and *S. paratyphi* isolated from typhoid fever patients were obtained from pathology laboratory of Murtala Muhammad Specialist Hospital, Kano. The isolateswere identified using different laboratory procedures including; Gram's stain, cultural characterization and Biochemical tests include (Indole, Methyl red, Voges Proskauer, and Citrate utilization) [22,23]. The isolates were maintained on Nutrient agar slants for further use.

### Collection of plant leaves and identification

The leaves of *A. indica* were collected at Karfi village, Kura Local Government Area in Kano State, Nigeria. The identification and authentication of the plant materials was done at the Herbarium in the Department of Biological Science, Bayero University Kano with the following voucher number BUKHAN 0312, and voucher specimens were deposited there for future reference. The leaves were washed thoroughly with distilled water and shade dry for 2 weeks. The dry leaves were grinded into powder using a sterile pestle and mortar under laboratory condition. The powder was kept in air tight container for future use as described by Ali et al. [24].

### Preparation of plant extracts

Methanol and water were used in the extraction process. Fifty grams (50 g) powder of the plant leaf was soaked in 500 mL each of distilled water and methanol respectively. The flasks were kept at room temperature for 3 days with intermittent shaking after which filtration was done using Whatman filter paper. The methanol extracts was evaporated at 50°C using rotary evaporator while the aqueous extract was evaporated at 40°C in water bath until dried extract samples were obtained. All the dried extract samples were dissolved in 10% DMSO separately to the final concentration of 200 mg/mL as a stock concentration. The stock solutions were stored in refrigerator at $4^0$C for further use [24].

### Qualitative phytochemical screening

The qualitative phytochemical screening of the leaf extract of *A. indica* was conducted to determine the presence of various phytochemical components such as terpenoids, flavonoids, alkaloids, steroid, phenol, anthraquinone, saponin and tannin using standard methods as described by Sofowora [25] and Trease and Evans [26].

### Antibacterial activity of the extracts

The antibacterial activity of the extracts against the isolates was determined using agar well diffusion method as described by Ali et al. [24] with slight modifications. The prepared bacterial suspension equivalent to 0.5 McFarland Standard (equivalent to 1.5 x $10^6$ CFU) was inoculated into sterile Mueller- Hinton agar medium in a sterile Petri-dish. A sterile 6 mm diameter sterile cork-borer was used to bore 5 wells into the agar medium at equidistance. The wells were then filled up with approximately 0.1mL of the extract solution at a concentration of 50, 100, 150 and 200 mg/L taking care to prevent spillage onto the surface of the agar medium. The plates were allowed to stand on the laboratory bench for 1 hour to allow proper diffusion of the extract into the medium after which the plates were incubated at $37^0$C for 24 hours, and thereafter the plates were observed for zones of inhibition and measured. Amoxicillin 100 mg/mL (Pal Pharmacy) was used as a positive control in the experiment. The experiment was conducted in triplicate and the average zone of inhibition was calculated.

### Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration of the extract was determined using broth dilution technique. Two fold serial dilutions of the extracts were prepared by adding 2mL of 200mg/mL of the extract into a test tube containing 2mL of Nutrient broth, thus producing solution containing 100mg/mL of the extract. The process continues serially up to test tube No. 5, hence producing the following concentrations; 100, 50, 25, 12.5, 6.25 mg/mL. Test tube Number 6 does not contain extracts and serve as negative

control. Exactly 0.5 mL of 0.5 McFarland equivalent standards of test organisms were introduced into the test tubes and incubated at $37^0$ C for 24 hours. After incubation the test tubes were observed for growth by checking for turbidity [10].

### Minimum bactericidal concentration (MBC)

Minimum Bactericidal Concentration of the extracts was determined using procedure of Ahmed and Beg [27].From each tube that did not show visible growth in the MIC, 0.1mL was aseptically transferred into extract free Mueller Hilton agar plates. The plates were incubated at 37°C for 24 hours. The MBC was recorded as the lowest concentration of the extract that had less than 99% growth on the agar plates.

## Result

### Phytochemical screening of the extracts

The phytochemical constituents of aqueous and methanol leaf extracts of *A. indica* are presented in the table below Table 1. The result showed that both aqueous and methanol leaf extracts contain alkaloids, tannin, anthraquinone, flavonoids, phenols and steroids. Terpenoids are present in aqueous leaf extracts but absent in methanol extract. On the other hand, glycoside and saponin were absent.

**Table 1:** Phytochemical Screening of Aqueous and Methanol Leaf Extracts of A. indica.

| S/N | Phytochemicals | Aqueous extract | Methanol Extract |
|---|---|---|---|
| 1 | Alkaloids | + | + |
| 2 | Flavonoids | + | + |
| 3 | Phenol | + | + |
| 4 | Terpenoid | + | - |
| 5 | Anthraquinone | + | + |
| 6 | Glycosides | - | - |
| 7 | Saponin | - | - |
| 8 | Steroids | + | + |
| 9 | Tannin | + | + |

Key: + = Present, - = Absent.

### Antibacterial activity of the extracts

The antibacterial activity of aqueous and methanol extracts of *A. Indica* against *S. typhi* and *S. paratyphi* is presented in Table 2. The result shows that highest antibacterial activity was demonstrated against *S. Typhi* with zone of inhibition of 21.70 mm at 200 mg/mL of methanol leaf extract. Zones of inhibition shown by control (100 mg/mL Ciprofloxacin) were 25.30 mm and 23.70 mm for *S. typhi* and *S. paratyphi* respectively.

### MIC and MBC of the extracts

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of aqueous and methanol extracts of *A. indica* against *S. typhi* and *S. paratyphi* is presented in presented in Table 3. The result shows that dilutions of various concentrations of the plant leaf extract are active against test isolates. The MIC ranges from 6.25 – 25 mg/mL while MBC ranges from 12.5 – 50 mg/mL.

**Table 2:** Antibacterial Activity of the Extracts against *S. typhi* and *S. paratyphi*.

| Extract | Concentration (mg/mL) | *S. typhi* | *S. paratyphi* |
|---|---|---|---|
| Aqueous leaf extract | 50 | 10.30±0.12 | 9.70±0.17 |
| | 100 | 12.30±0.07 | 10.30±0.20 |
| | 150 | 15.70±0.09 | 13.30±0.09 |
| | 200 | 19.70±0.10 | 15.70±0.06 |
| Control | | 25.3±0.20 | 23.7±0.14 |
| | 50 | 12.30±0.21 | 10.70±0.10 |
| Methanol leaf extract | 100 | 16.70±0.16 | 15.30±0.07 |
| | 150 | 17.30±0.07 | 15.70±0.12 |
| | 200 | 21.70±0.09 | 18.30±0.08 |

**Table 3:** MIC and MBC of the Extracts.

| Extracts | *S. typhi* | *S. paratyphi* |
|---|---|---|
| Aqueous Leaf Extract | 12.5/50 | 25/50 |
| Methanol Leaf Extract | 12.5/12.5 | 12.5/25 |

## Discussion

The presence of secondary metabolites in plant lead to production of some biological activity of the plant in man and it is responsible for their use as herbs [10]. These compounds also serve to protect the plant against infection by microorganisms, predation by insects and herbivores, while some give plants their odors and /or flavors and some still are responsible for their pigments [2]. 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 [28].

The result of the present study revealed the present of phytochemical compound in the leaf extracts of *A. indica*. This result was in conformity with the result of several findings conducted by many researchers [29]. *A. indica* leaf contains several valuable components such as alkaloid, flavonoid and isoprenoids that include terpenoids containing limonoids, azadirone and its derivatives [13].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 [30]. Some of these metabolites particularly the flavonoids, tannin and alkaloid were reported to be responsible for antimicrobial activity associated with some ethno-medicinal plant [31].

The antibacterial property of the extracts against the test isolates revealed that *S. Typhi* showed the highest zones of inhibition (23 mm) than *S. paratyphi*. This result justifies the finding of Al-Akel *et al*. [28]. The leaf extracts of the plant is known

to possess antibacterial activity against pathogenic bacteria such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella typhi* and *Bacillus pumillas* [12]. The antimicrobial activity of the leaf extracts is attributed to the presence of phytochemical constituents in the plant's leaves. The methanol extract was found more active than aqueous extract and this justified several studies conducted involving aqueous and organic extract [10,24,32] since most studies have reported that organic solvents were better chemical reagents for consistent extraction of antimicrobial substances from medicinal plants. The test organisms had the same minimum inhibitory concentration (MIC) value ranges of 6.25 to 25 mg/mL and minimum bactericidal concentration (MBC) value ranges of 12.5 to 50 mg/mL. This indicated that the leaves *A. indica* possessed antibacterial property against *S. typhi and S. paratyphi*. This is similar to the findings of the National Library of Medicine at the National Institutes of Health (www.pubmed.com) who reported that in test tubes, *A. 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.

## Conclusion

It may be concluded from this study that *A. indica* leaf extracts have antibacterial activity against *S. typhi and S. paratyphi* isolated from typhoid fever patients. The antibacterial activity is probably due to the presence of phytochemical constituents such as alkaloids, tannin, anthraquinone, flavonoids, phenols and steroids. The wide use of *A. indica* is attributable to the presence of these bioactive compounds, which may explain its many traditional uses against typhoid fever.

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## Conflict of Interest

No Conflict of Interest.

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