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Phytochemical Analysis and Antibacterial Activity of *Azadirachta indica* Leaf Extracts against *Escherichia coli*

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Abstract

Background: Traditional medicine has employed *Azadirachta indica* to treat a variety of ailments. However, there is little information on the use of this plant in Zambia. **Objective:** To evaluate the phytochemicals and antibacterial activity of *Azadirachta indica* leaf extracts against *Escherichia coli*. **Materials and Methods:** This was a laboratory-based experimental study in which ethanol and water were used for extraction by maceration. Phytochemical analysis was then done on the leaf extract. Using the disc diffusion method, varying concentrations of *A. indica* aqueous and ethanolic extracts were used to test the antibacterial activity of *A. indica against Escherichia coli*. The sensitivity of the tested microorganisms to aqueous and ethanolic leaf extracts was shown by zones of inhibition after incubation. **Results:** The phytochemical screening of the *A. indica* leaves revealed the presence of phenolics and tannins in both the ethanol and aqueous extract. Saponins, flavonoids and alkaloids were only present in the aqueous extract. While steroids were only present in the ethanol extract. The antibacterial activity of *A. indica* leaves extract was determined by zones of inhibition which showed that both aqueous and ethanol extracts had activity against *E. coli*. The Minimum Inhibitory Concentration was determined at 10 mg/mL for the aqueous extract and 20 mg/mL for the ethanol extract. The zones of inhibition increased with concentration. **Conclusion:** The extracts of *A. indica* displayed

antibacterial activity against *E. coli* in a dose-dependent manner. Comparatively, the aqueous extract produced better antibacterial properties against *E. coli* than the ethanolic extract.

Keywords

Phytochemicals, Antibacterial Activity, *Escherichia coli*, *Azadirachta indica*, Neem

1. Introduction

Infectious diseases continue to be the major cause of mortality in Africa [1]. Well known existing, emerging, and re-emerging diseases like malaria, tuberculosis, HIV/AIDS, cholera, and others are causing suffering and mortality to a wide population in developing countries especially Africa [2].

Escherichia coli (*E. coli*) is one of the most frequent causes of many common bacterial infections [3]. However, the treatment of *E. coli* infections is threatened by the emergence of antimicrobial resistance [4] [5] [6]. According to recent studies, *E. coli* isolates show high rates of resistance to conventional drugs like erythromycin, amoxicillin, and tetracycline [7] [8].

Antibiotic resistance is a severe and developing public health issue that poses a global health concern [9]. Infectious diseases are becoming more difficult to treat due to multi-drug resistant bacteria especially Gram-positive pathogens [10] [11]. These problems are of even greater relevance to rural areas of Zambia, where the choice of antibacterial agents is rather limited due to limited resources. In these areas, the most practical option remains to search for cheap alternatives like traditional medicine for their primary health care needs to manage bacterial infections [12].

Azadirachta indica plant possesses several medicinal properties which include antibacterial activity, antioxidant effect, anti-inflammatory effect, anti-cancerous effect, hepatoprotective, anti-malarial, anti-diabetic, anti-HIV/AIDS, and anti-hypertensive effect activity [13] [14]. These activities are a result of the phytochemicals present in the plant [15]. Therefore, this study aimed at evaluating the phytochemicals and antibacterial activity of *A. indica* leaf extracts against *E. coli*.

2. Materials and Methods

2.1. Collection of Plant Material

The A. *indica* leaves were collected from Lusaka, Zambia in September 2021 and the study was conducted from 15th September to 15th October 2021. A voucher herbarium specimen was prepared and botanical identification and authentication were done at the University of Zambia (UNZA), School of Natural Sciences, Department of Biological Sciences in the Herbarium section.

2.2. Preparation of the Plant Extracts

The leaves of *A. indica* were removed loosely from the plant stem by the use of a knife. The leaves were washed under running tap water to eliminate dust and other foreign particles and dried under a shade for 7 days. The dried leaves were then ground into a powder using a mortar and pestle. 50 g of the coarse powder was extracted with 750 mL of either 95% EtOH or distilled water by intermittent shaking for 3 days. The mixtures were filtered using Whatman® number one filter paper to obtain clear filtrates. The resulting filtrates were then dried in beakers using a water bath at 60˚C for approximately 12 hours to afford 4.84 g of an ethanolic extract and 11.1 g of an aqueous extract. The extracts were then covered and stored in a refrigerator for further use [5].

2.3. Calculation of Percentage Yield

The percentage extractive yield was calculated using the formula below [5].

% Extraction yield (w/w) = weight of extraction recovered/initial weight of powder × 100.

The retained weights were 4.84 g Ethanol extract and 11.10 g aqueous extract.

2.4. Phytochemical Analysis

2.4.1. Sodium Hydroxide Test for Flavonoids

0.5 g of the ethanolic and aqueous extract was dissolved in water and filtered; to this, 2 ml of the 10% aqueous sodium hydroxide was later added to produce a yellowish discolouration. A change in colour from yellow to colourless on the addition of dilute hydrochloric acid indicated the presence of flavonoids [16].

2.4.2. Ferric Chloride Test for Phenolics

0.5 g of the ethanolic and aqueous extract was mixed with distilled water and then filtered. To 2 ml of the filtrate, a few drops of 10% ferric chloride solution were then added. A green-blue or violet colouration indicated the presence of a phenolic hydroxyl group [16].

2.4.3. Test for Tannins

0.5 g of the ethanolic and aqueous extract was stirred with about 10 mL of distilled water and then filtered. A few drops of 1% ferric chloride solution were added to 2 ml of the filtrate. The occurrence of a blue-black, green or blue-green precipitate indicates the presence of tannins [16].

2.4.4. Test for Saponins

1 g of the ethanolic and aqueous extract was mixed with 5 mL of distilled water then filtered. To the filtrate, about 3 mL of distilled water was further added and shaken vigorously for about 5 minutes. Frothing which persisted on warming was taken as evidence for the presence of saponins [17].

2.4.5. Test for Alkaloids

0.5 g of the ethanolic and aqueous extract was stirred with 5 mL of the water and

then filtered. Of the filtrate, 3 mL was taken individually into a test tube. To the first portion, a few drops of Wagner's reagent were added and agitated; the occurrence of reddish-brown precipitate was taken as positive [17].

2.4.6. Test for Terpenoids

0.5 g of the ethanolic and aqueous extract was dissolved in ethanol. To it, 1 mL of acetic anhydride was added followed by the addition of concentrated sulphuric acid. A change in colour from pink to violet showed the presence of terpenoids [17].

2.4.7. Liebermann-Burchard Test for Steroid

To 0.2 g of the ethanolic and aqueous extract, 2 mL of acetic acid was added; the solution was cooled well in ice followed by the addition of concentrated sulphuric acid carefully. Colour development from violet to blue or bluish-green indicated the presence of a steroidal ring *i.e.* aglycone portion of cardiac glycoside [17].

2.5. Culturing and Collection of Bacteria

E. coli (ATCC 25922) was cultured in the microbiology laboratory, Department of Pathology and Microbiology at the University Teaching Hospitals (UTH), Lusaka, and collected for experimentation.

2.6. Preparation of Bacterial Isolates

The bacteria were incubated in a controlled acidic environment at 37˚C with the proper amount of food to promote bacterial growth [18].

2.7. Inoculum Preparation

This investigation used the direct state suspension method; therefore, turbidity was contrasted using clean saline and 0.5 McFarland turbidity principles. The turbidity was balanced with saline until it coordinates that of 0.5 McFarland turbidity measures. This was finished by holding the suspension and the 0.5 McFarland turbidity gauges before a light source against a white foundation with differentiating dark lines [19].

2.8. Inoculation

The Muller-Hinton agar plates were utilized for *E. coli*. Before inoculation, care was taken to ensure that there was no excessive wetness on the agar surface and that the plates were not excessively dry (wrinkled surface demonstrates excessive dryness). A sterile cotton swab was placed into the microscopic organisms' suspension. Abundance inoculum was expelled by passing the swab inside the microscopic organisms' suspension tube. The media was at that point inoculated by swabbing the agar surfaces in two ways at a 90-degree edge to each surface and the third line at a 45-degree edge and afterwards it was permitted to represent 20 minutes to encourage assimilation of abundance inoculum before use of the test

plant removes [20].

2.9. Disc Diffusion Method

The disc diffusion method was used to test the antibacterial properties. The disc was dipped in the extract and the extract left to diffuse in the disc and then placed on the agar media containing the bacteria [21] [22]. Ciprofloxacin 5 µg standard discs were used as the positive control against *E. coli* while dimethyl sulphoxide (DMSO) and sterile water were used as a negative control to compare the results with that of experimental ethanol and water extracts respectively.

2.10. Antimicrobial Activity Determination

The different concentrations (1 mg/mL, 10 mg/mL, 20 mg/ml, 30 mg/mL, 40 mg/ml, and 50 mg/mL) of the aqueous and ethanolic extracts of *A. indica* were tested on the culture strains of *E. coli* on agar plates. The sensitivity of the tested pathogenic organisms to aqueous and ethanolic extracts was shown by zones of inhibition after incubation. The zones of inhibition were measured using a plastic ruler in millimetres. For each concentration of the extract, the zone of inhibition was measured three times to minimize the error and the mean was recorded. The statistical analysis was then performed. The Minimum Inhibitory Concentration (MIC) for the crude extract was determined by the agar-disc diffusion method. MIC was determined as the lowest concentration at which a clear zone of microbial growth inhibition will be observed [23].

2.11. Data Analysis

Microsoft excel 2013 was used to process collected data. Descriptive statistics on the phytochemical composition of the extract was presented in form of a table. ANOVA was performed to compare the activity of different concentrations of the extract and the control drug on *E. coli*. Results were presented in form of tables. All statistical analyses were considered significant at a 95% confidence level and $p < 0.05$.

3. Results

3.1. Percentage Extraction Value

Following aqueous and ethanolic extractions of the leaf, the percentage extraction value of *Azadirachta indica* extracts were 22.2% and 9.75% respectively. See **Table 1**.

3.2. Phytochemical Analysis

Following phytochemical analysis of the ethanolic and aqueous extracts of *A. indica*, phenolic compounds, tannins and steroids were present in the ethanolic extract while, flavonoids, phenolic compounds, tannins, saponins and alkaloids were present in the aqueous extract. See **Table 2**.

Table 1. Percentage extraction yield of aqueous and ethanolic *A. indica* extracts.

Type of solvent	Initial Weight of the leaf in grams	Extractive value	Percentage extraction value
Ethanol	50	4.85	9.75%
Water	50	11.1	22.2%

Table 2. Phytochemical analysis of *Azadirachta indica* aqueous and ethanolic extracts.

Phytochemicals tested	Aqueous extract	Ethanolic extract
Flavonoids	Present	Absent
Phenolics	Present	Present
Tannins	Present	Present
Saponins	Present	Absent
Alkaloids	Present	Absent
Terpenoids	Absent	Absent
Steroids	Absent	Present

3.3. Antibacterial Activity of Aqueous and Ethanolic Extracts of *A. indica* against *E. coli*

The mean zone of inhibition produced at different concentrations of both ethanolic and aqueous extracts of *A. indica* against *E. coli* showed that the aqueous extract at 50 mg/ml had the highest zone of inhibition (10.67 ± 0.58 mm) compared to (8.7 ± 0.58 mm) for the ethanolic extract (p-value = 0.072). Ciprofloxacin's (control) mean zone of inhibition was 34.67 ± 0.58 mm (p-value = 0.025). The minimum inhibitory concentration (MIC) against *E. coli* was 10 mg/ml for the aqueous extract and 20 mg/ml for the ethanolic extract. See Table 3.

4. Discussion

The medicinal values of the secondary metabolites are because of chemical substances that produce a definite physiological action on the human body. These chemical substances include alkaloids, glycosides, steroids, flavonoids, fatty oils, resins, mucilage, tannins, gums, phosphorus and calcium for cell growth, replacement and bodybuilding [24].

In this study, the *A. indica* leaf extracts contained the following phytochemicals; flavonoids, phenolics, tannins, saponins, alkaloids and Steroids. Phenolics and Tannins were observed in different intensities in the aqueous and ethanolic extracts. On the one hand, the aqueous extract showed the presence of all the phytochemicals listed above except for steroids and terpenoids. However, the ethanolic extract only showed the presence of tannins, phenolics and steroids. The results observed in this study are similar to results reported by Sahrawat *et al*, who reported the presence of saponins in the aqueous extract and phenolics and tannins in the ethanol extract of *A. indica* growing in India [25]. Unlike in this study where terpenoids were absent in both the aqueous and ethanolic extract, Gupta and colleagues reported the presence of terpenoids in both the

Table 3. Mean zone of inhibition of aqueous and ethanolic extracts against *E. coli*.

Concentration of extract	Aqueous extract Mean zone of inhibition (mm)	Ethanolic extract Mean zone of inhibition (mm)	Ciprofloxacin (control) mean zone of inhibition (mm)
1 mg/mL	0	0	34.67 ± 0.58
10 mg/mL	2.33 ± 2.08*	0	
20 mg/mL	5.7 ± 0.58	6.67 ± 0.58*	
30 mg/mL	8.78 ± 0.58	5.67 ± 0.58	
40 mg/mL	10.00 ± 1.00	7.67 ± 0.58	
50 mg/mL	10.67 ± 0.58	8.7 ± 0.58	

*- MIC of aqueous and ethanolic extracts against *E. coli*.

aqueous and ethanol extract from a plant growing in India [26]. Steroids were only present in the *A. indica* ethanolic extract similar to results reported by Susmitha *et al.* who also found steroids and tannins in the ethanolic extract [27]. The observed difference in phytochemical presence can be attributed to differences in the polarities of the solvents. Furthermore, due to geographical and environmental factors that the plant is exposed to, studies in other parts of the world may report the presence or absence of phytochemicals reported in this study.

The highest concentration of 50 mg/ml of both aqueous and ethanolic extracts showed an average zone of inhibition of 10.67 ± 0.58 mm and 8.7 ± 0.58 mm respectively while ciprofloxacin 5 µg the positive control produced an average zone of inhibition of 35 mm with p-value = 0.025. The observed statistically significant difference among the three results is because ciprofloxacin was in its purest antimicrobial form, containing 100% active ingredient. There was no statistically significant difference between the aqueous and ethanolic extracts at 50 mg/ml (p-value = 0.072). However, the aqueous extract at different concentrations showed statistically significant superiority over the ethanolic extract (p-value = 0.004). Unlike data reported in this study, Gupta *et al* reported that the ethanolic extract at 100 mg/mL had more activity against *E. coli* with a zone of inhibition of 1.2 cm compared to the aqueous extract at 100 mg/mL which reported a zone of inhibition of 0.8 cm [26]. Similarly, Susmitha *et al.* showed that ethanolic extract activity against *E. coli* was 8 mm while the aqueous extract had no activity [27]. However, the observed difference in inhibitory zones could be due to the difference in phytochemical presence in the extracts since solvents of different polarities were used. Different polarities as demonstrated in this study affected the types of phytochemicals extracted and hence the therapeutic effects of the plant extract. Further, observed differences among the studies reported could be as a result of variations in geographical regions, rain patterns, and harvesting seasons.

The minimum inhibitory concentration (MIC) of *A. indica* aqueous extract was found to be 10 mg/mL while the ethanolic extract was 20 mg/mL. In a study

conducted by Nigussie *et al.,* that the MIC for *A. indica* methanolic extract against *E. coli* was reported as 83.3 ± 29.0 mg/ml [28]. However, a study by Shu'aibu *et al.* determined the MIC of the petroleum ether extract of *A. indica* at 100 µg/mL against *E. coli* while the ethanolic and methanolic extracts had no activity at that concentration [29]. This shows that *A. indica* activity is significantly affected by the polarity of the solvent used for extraction.

5. Limitations

The antibacterial activity of *A. indica* against *E. coli* reported in this study cannot be generalized to other microorganisms. This is because the tests were conducted strictly on *E. coli*. Besides, other plant parts such as stems and roots may have different antibacterial activities.

6. Conclusion

A. indica aqueous and ethanolic extract displayed antibacterial activity against *E. coli*. The aqueous extract showed better antibacterial activity than the ethanolic extract. The antibacterial activity of *A. indica* extracts was concentration-dependent as observed from the varying zones of inhibition with change in concentration.

7. Recommendations

The authors recommend that a bio guided fractionation be done to determine the phytochemicals that cause the antibacterial activity observed. Besides, future studies should be focused on assessing the antibacterial activity of the Neem plant on multiple microorganisms using different parts such as stems, roots and leaves.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Nachega, J., Seydi, M. and Zumla, A. (2020) The Late Arrival of Coronavirus Disease 2019 (COVID-19) in Africa: Mitigating Pan-Continental Spread. *Clinical Infectious Diseases*, **71**, 875-878. https://doi.org/10.1093/cid/ciaa353

[2] Kindhauser, M.K. and WHO (2003) Communicable Diseases 2002: Global Defence against the Infectious Disease Threat. World Health Organization.

[3] Madappa, T. (2012) *Escherichia coli* Infections. Emedicine Medscape. http://emedicine.medscape.com/article/217485-overview#a0101

[4] Holmes, A.H., Moore, L.S., Sundsfjord, A., Steinbakk, M., Regmi, S., Karkey, A., *et*

al. (2016) Understanding the Mechanisms and Drivers of Antimicrobial Resistance. *The Lancet*, **387**, 176-187. https://doi.org/10.1016/S0140-6736(15)00473-0

[5] Masaiti, G.C., Malambo, C., Hikaambo, C., Banda, M., Matafwali, S.K., Mufwambi, W., *et al.* (2019) Antibacterial Properties of *Ficus sycomorus* Bark Extract against *Staphylococcus aureus* and *Escherichia coli*. *International Journal of Biomedical Investigation*, **2**, 1-7. https://doi.org/10.31531/2581-4745.1000121

[6] Allocati, N., Masulli, M., Alexeyev, M.F. and Di Ilio, C. (2013) *Escherichia coli* in Europe: An Overview. *International Journal of Environmental Research and Public Health*, **10**, 6235-6254. https://doi.org/10.3390/ijerph10126235

[7] Apenteng, J.A., Osei-Asare, C., Oppong, E.E., Amihere, I. and Hafiz, M.Y. (2017) Antibiotic Sensitivity Patterns of Microbial Isolates from Fish Ponds: A Study in the Greater Accra Region of Ghana. *African Journal of Pharmacy and Pharmacology*, **11**, 314-320. https://doi.org/10.5897/AJPP2017.4789

[8] Kibret, M. and Abera, B. (2011) Antimicrobial Susceptibility Patterns of *E. coli* from Clinical Sources in Northeast Ethiopia. *African Health Sciences*, **11**, 40-45. https://doi.org/10.4314/ahs.v11i3.70069

[9] Aslam, B., Wang, W., Arshad, M.I., Khurshid, M., Muzammil, S., Rasool, M.H., *et al.* (2018) Antibiotic Resistance: A Rundown of a Global Crisis. *Infection and Drug Resistance*, **11**, 1645-1658. https://doi.org/10.2147/IDR.S173867

[10] Morris, S. and Cerceo, E. (2020) Trends, Epidemiology, and Management of Multi-Drug Resistant Gram-Negative Bacterial Infections in the Hospitalized Setting. *Antibiotics*, **9**, Article No. 196. https://doi.org/10.3390/antibiotics9040196

[11] Mazzariol, A., Bazaj, A. and Cornaglia, G. (2017) Multi-Drug-Resistant Gram-Negative Bacteria Causing Urinary Tract Infections: A Review. *Journal of Chemotherapy*, **29**, 2-9. https://doi.org/10.1080/1120009X.2017.1380395

[12] Chinsembu, K. (2016) Ethnobotanical Study of Medicinal Flora Utilised by Traditional Healers in the Management of Sexually Transmitted Infections in Sesheke District, Western Province, Zambia. *Revista Brasileira de Farmacognosia*, **26**, 268-274. https://doi.org/10.1016/j.bjp.2015.07.030

[13] Maleki, L., Sadeghian-Rizi, T., Ghannadian, M., Sanati, M.H., Shafizadegan, S. and Sadeghi-Aliabadi, H. (2017) Antibacterial Activity of *Azadirachta indica* Leaf Extracts against Some Pathogenic Standards and Clinical Bacterial Isolates. *Avicenna Journal of Clinical Microbiology and Infection*, **5**, Article ID: 12987. https://doi.org/10.5812/ajcmi.12987

[14] Pandey, G., Verma, K. and Singh, M. (2014) Evaluation of Phytochemical, Antibacterial and Free Radical Scavenging Properties of *Azadirachta indica* (Neem) Leaves. *International Journal of Pharmacy and Pharmaceutical Sciences*, **6**, 444-447.

[15] Hashmat, I., Azad, H. and Ahmed, A. (2012) Neem (*Azadirachta indica* A. Juss)—A Nature's Drugstore: An Overview. *International Research Journal of Biological Sciences*, **1**, 76-79.

[16] Evans, W.C. (2002) Trease and Evans' Pharmacognosy. WB Saunders, New York, 553-557.

[17] Ogbeba, J., Iruolaje, F. and Dogo, B. (2017) Antimicrobial Efficacy of *Guiera senegalensis* and *Prosopis africana* Leave Extract on Some Bacterial Pathogens. *European Journal of Biology and Medical Science Research*, **5**, 27-36.

[18] Beyene, G. and Tsegaye, W. (2011) Bacterial Uropathogens in Urinary Tract Infection and Antibiotic Susceptibility Pattern in Jimma University Specialized Hospital, Southwest Ethiopia. *Ethiopian Journal of Health Sciences*, **21**, 141-146. https://doi.org/10.4314/ejhs.v21i2.69055

[19] Idelevich, E.A., Storck, L.M., Sparbier, K., Drews, O., Kostrzewa, M. and Becker, K. (2018) Rapid Direct Susceptibility Testing from Positive Blood Cultures by the Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry-Based Direct-on-Target Microdroplet Growth Assay. *Journal of Clinical Microbiology*, **56**, e00913-e00918.

[20] Brown-Elliott, B.A., Nash, K.A. and Wallace, R.J. (2012) Antimicrobial Susceptibility Testing, Drug Resistance Mechanisms, and Therapy of Infections with *Nontuberculous mycobacteria*. *Clinical Microbiology Reviews*, **25**, 545-582.

[21] Abalkhil, T.A., Alharbi, S.A., Salmen, S.H. and Wainwright, M. (2017) Bactericidal Activity of Biosynthesized Silver Nanoparticles against Human Pathogenic Bacteria. *Biotechnology & Biotechnological Equipment*, **31**, 411-417. https://doi.org/10.1080/13102818.2016.1267594

[22] Bashiti, T.A., Elmanama, A.A. and Masad, A.A. (2011) The Antibacterial and Synergistic Effects of Some Palestinian Plant Extracts on *Escherichia coli* and *Staphylococcus aureus*. *Functional Plant Science and Biotechnology*, **5**, 57-62.

[23] Margaret, P., Duo, L., Tengfei, L. and Andrew, B. (2020) *Berberine* verses *Sorafenib* for the Treatment of *Hepatocellular carcinoma*. *International Journal of Biomedical Investigation*, **3**, 1-9. https://doi.org/10.31531/2581-4745.1000125

[24] Kubmarawa, D., Khan, M., Punah, A. and Hassan, M. (2008) Phytochemical Screening and Antimicrobial Efficacy of Extracts from *Khaya senegalensis* against Human Pathogenic Bacteria. *African Journal of Biotechnology*, **7**, 4563-4566.

[25] Sahrawat, A., Sharma, J., Rahul, S., Tiwari, S., Joshi, M. and Pundhir, A. (2018) Phytochemical Analysis and Antibacterial Properties of *Azadirachta indica* (Neem) Leaves Extract against *E. coli*. *Journal of Pharmacognosy and Phytochemistry*, **7**, 1368-1371.

[26] Gupta, A.K., Ahirwar, N.K., Shinde, N., Choudhary, M., Rajput, Y.S. and Singh, A. (2013) Phytochemical Screening and Antimicrobial Assessment of Leaves of *Adhatoda vasica*, *Azadirachta indica* and *Datura stramonium*. *Pharmaceutical and Biosciences Journal*, **1**, 42-47. https://doi.org/10.20510/ukjpb/1/i1/91118

[27] Susmitha, S., Vidyamol, K., Ranganayaki, P. and Vijayaragavan, R. (2013) Phytochemical Extraction and Antimicrobial Properties of *Azadirachta indica* (Neem). *Global Journal of Pharmacology*, **7**, 316-320.

[28] Nigussie, D., Davey, G., Legesse, B.A., Fekadu, A. and Makonnen, E. (2021) Antibacterial Activity of Methanol Extracts of the Leaves of Three Medicinal Plants against Selected Bacteria Isolated from Wounds of Lymphoedema Patients. *BMC Complementary Medicine and Therapies*, **21**, Article No. 2. https://doi.org/10.1186/s12906-020-03183-0

[29] Shu'aibu, I., Hamman, J., Goje, L., Mu'inat, A., Jauro, H. and Kabiru, M. (2015) Phytochemical Analysis and Evaluation of Bacteriostatic Effect of Neem (*Azadirachta indica*) Leaves on Some Clinical Bacterial Isolates. *Journal of Harmonized Research in Applied Sciences*, **3**, 152-157.