Global Academic Journal of Pharmacy and Drug Research

Available online at https://www.gajrc.com
DOI: 10.36348/gajpdr.2024.v06i01.002

OPEN ACCESS

ISSN (P): 2706-9044
ISSN (O): 2707-255X

Original Research Article

Investigating the Efficacy of Neem Leaves as Mosquito Repellent

Kamilu B. S.[1*], Abdulrahman Umar[2], Zubairu A. Y.[3,5], Egwuonwu K. C.[1], Dahiru M. A. Umar[3], Muhammad Sani[4]

[1]Department of Biology, Federal College of Education Gidan Madi Sokoto State
[2]Department of Chemistry, Federal College of Education Gidan Madi Sokoto State
[3]Department of Science Laboratory Technology, Umaru Ali Shinkafi Polytechnic Sokoto
[4]Department of Pure Science, Sultan Muhammadu Maccido Institute of Qur'an and Islamic Studies Sokoto
[5]Department of Pure and Applied Chemistry Usmanu Danfodiyo University Sokoto P.M.B 2346 Nigeria

***Corresponding Author**
Kamilu B. S.
Department of Biology,
Federal College of Education
Gidan Madi Sokoto State

Article History
Received: 02.01.2024
Accepted: 07.02.2024
Published: 16.02.2024

Abstract: Herbal plants might have a great potential to be used as antimicrobial agents. Ethanolic leaf extracts of neem were investigated for the presence of phytochemical, physicochemical and their antimicrobial activities at various concentrations against some selected clinical microbes (*Escherichia coli* and *Salmonella typhi*) using standard methods. Qualitative phytochemical results showed that the ethanolic leaf extracts contained phytochemicals constituents such as alkaloids, falavonoids, terpenoids and saponin were assayed except glycosides, phenols and tannins were all absent in the extracts of neem. The physicochemical parameters of the extract revealed the pH to be 6.42 while the iodine value was 0.634 and the electric conductivity was 48.2. The results of the antibacterial activities of the ethanolic extract of neem leaves were effective on the tests organisms (*Escherichia coli* and *Salmonella spp*) at various concentrations and also sensitive against cefraxone at same concentration. In this study, neem ethanolic extracts exhibited varying levels of antibacterial activity against *Salmonella spp* and *Escherichia coli.* The antimicrobial activity of neem extract varies widely, depending on the type of herb on the test medium, and microorganism. For these reasons, neem leaf might not be considered as excellent antimicrobials agents.

Keywords: *Escherichia coli*, Neem, Mosquito, *Salmonella typhi*.

1.0 INTRODUCTION

Mosquitoes are one of the most common vectors for transmitting diseases such as malaria, dengue fever, yellow fever, and Zika virus. The use of chemical-based insecticides and repellents has been the most important method of controlling mosquito populations. However, these chemicals have detrimental effects on the environment as well as human health thus, there is a necessity to explore alternative methods for mosquito control (Green *et al.,* 2002).

Neem seed and leaves have been used traditionally as a natural mosquito repellent. The active ingredients in neem have been found to have insecticidal and repellent properties (Alam *et al.,* 2009). This study aims to investigate the efficacy of neem leaves as a mosquito repellent.

In many ethno botanical evaluation plants acts as repellent agents, in various part of the world plant repellents ware widely used. Plant based repellents do not pose affect domestic animals as well as humans moreover easily biodegraded when compare to the synthetic compounds, natural products are safer for human use, too much uses of synthetic repellents may causes insecticide resistance in mosquitoes, harmful effect on non-

target organisms and has threat for the environment (Irving *et al.,* 2005).

Due to the less impact on environment and less economical cost, plant based repellents attracted great attention of people. Innovative drug distribution systems of plant based active constituents are need of the time. Modern technologies in standardization and isolation of herbal drugs motivation are necessary. Mosquito repellent devices such as nets of different types are also necessary for trapping of mosquitoes (Burt and Reinders, 2003).

1.1 Statement of the Problems/Justification

Processing of neem seed and leaves into essential oils is a sure way of transforming these wastes with great potential for environmental pollution into a resource with great potential for economic prosperity, and also for securing the public health impacts of safer and healthier environment, likely to be obtained from the indirect waste management option so offered. This study therefore, aims to lessen the waste disposal of neem seed and leaves and to propose an alternative insecticide/repellent that is less harmful than the synthetic chemical products, but conforms to the standards of the market (Burt and Reinders, 2003).

Recent studies showed that the smoke generated from burning mosquitoes coil is of certain health concerns. A person being exposed to the smoke coming from the coil may suffer severe headache, nausea and vomiting, the condition will be severe among asthmatic patients. The emission from one burning coil can be as high as that released from 51 burning cigarettes. This is because of the chemicals found in mosquito coils out of natural ingredients may remove these problems.

1.2 Objectives of the Study

The specific objectives were to;

i. Extract the active ingredients from neem leaf
ii. Convert the leaf waste to useful product
iii. Characterized the active ingredients in the extracts
iv. Characterize the physicochemical parameters of the extracts
v. Determine the antibacterial activity of the extract against *Salmonella spp* and *Escherichia coli*.

2.0 MATERIALS AND METHODS

2.1 Materials

2.1.1 Collection of Plant Samples: The plant samples were collected from Gidan Madi Town, Tangaza Local Government Area, Sokoto State. The neem leaves were authenticated at Department of Science Laboratory Technology, Umaru Ali Shinkafi Polytechnic Sokoto

Fig. 1: Neem (*Azadirachta indica*) species (Source: Self collection from study site)

2.1.2 Preparation of Plant Samples: The neem leaves were cut into bits with knife and oven dried at 70°C for 12 h to remove all moisture. The samples were then ground into fine powder.

2.1.3 Materials and Instruments

Neem leaves, 8 meters plate, Muller Hinton Agar, 10 centimeters glass tube, What man filter paper, 5ml and 10ml syringe, mortar and pistle.

2.2 Methods

2.2.1 Extraction of Plant Material

The ethanolic extract of the plant was prepared by soaking 50g of the ground sample of the leaf in 100 mL of ethanol using maceration process for 48hours for a complete maceration. The experimental set-up was left for 48 h at room temperature and thereafter filtered using Whatman filter paper No. 1. The extract was then concentrated to 50 mL of the original volume of the extract and

stored in an air tight container in a refrigerator at 4°C until when needed.

2.2.2 Preliminary Phytochemical Screening:

Qualitative phytochemical screening of the extracts was conducted to determine the presence of phytochemicals such as tannins, saponins, flavonoids, alkaloids, sterols, phenols and cyanogenic glycoside. This was done using standard procedure as described by Harborne (1973).

2.3 Physicochemical Parameters:

2.3.1 Iodine Test

This test was performed to check the presence of unsaturated bonds in a molecule. A few drops of the neem extract were taken in a test tube. A few crystals of iodine was added and shaken well.

2.3.2 *p*H Test

The *p*H was measured using pH meter. Approximately 5 mls of the neem extract was taken for *p*H analysis.

2.3.3 Electricity Conductivity

Kerro meter was inserted into the neem extract select the button for the parameter (Electric Conductivity) you want to detect and wait for the result to display on the screen to take your reading. Hannan instrument (Model: Hi-9828) was used to measure electrical conductivity.

2.4 Antibacterial Screening

2.4.1 Bacteria Used in the Study

The organisms (*Escherichia coli* and *Salmonella spp*) isolates used in this study were isolated from wound, nose and ear swabs and confirmed based on their cultural and biochemical characteristics.

2.4.2 Procedures of making the crude extract

Neem leaves were collected and room-dried, pulverised in a wooden mortar and weighed on an electronic weighing balance. Fifty (50 g) of the powder was extracted in ethanol using Soxhlet extraction method. The extract was filtered through Whatman (No.1) filter paper and concentrated over a water bath using a rotary-vacuum evaporator to recover the solvents. The extract was then stored for antibacterial activity studies.

2.4.3 Preparation of Culture Media

The media used were Mueller Hinton agar and Nutrient broth. The media were prepared according to manufacturer‟s instruction. Thirty five (35 g) of medium was mixed with one litre of distilled water in a screw cap container and autoclaved at 121 °C for 15 minutes. The medium was later dispensed into 90 mm sterile agar plates and left to set. The agar plates were incubated for 24 hours at 37 °C to confirm sterility.

2.4.4 Antibacterial Susceptibility test of the Plant Extracts

The susceptibility test of the extract was carried out using the agar well diffusion method (Irobi *et al.*, 1994). *Escherichia coli* and *Salmonella spp* were inoculated separately on the surface of Mueller Hinton agar plates by surface spreading using a sterile cotton swab and each bacterium evenly spread over the entire surface of agar plate to obtain a uniform inoculums (Irobi *et al.*, 1994). Five wells of 6 mm diameter and 5 mm depth were made on the solid agar on each plate using a sterile glass borer and numbered for the concentrations of extracts. 1g of the extract was dissolved in 10 ml of distilled water to obtain 2, 4, 6 and 8 mg/ml concentration. The set up was incubated for 24 hours at 37^{o}C. All the tests were run in triplicates. After incubation, the zones of inhibition were measured in millimeters (mm) using a transparent ruler. Oxoid (1985) standard susceptibility range was used to classify zones of inhibition as either sensitive.

3.0 RESULTS AND DISCUSSION

3.1 Results

The results of the study are presented in Table 1-3 and Fig. 1. Qualitative phytochemical screening of leaf extracts of neem ethanolic solvents is presented in Table 1. It was observed from the result that phytochemical such as alkaloids, flavonoids, terpenoids and saponin were assayed except glycosides, phenols and tannins were all absent in the extracts of neem (Table 1). The physicochemical parameters of the extract revealed the pH to be 6.42 while the iodine value was 0.634 and the electric conductivity was 48.2 as presented in table 2. The results of the antibacterial activities of the ethanolic extract of neem leaves were slightly effective on the tests organisms (*Escherichia coli* and *Salmonella spp*) at various concentrations and also sensitive against cefraxone at same concentration. The results are presented in table 3.

Table 1: Qualitative Phytochemical Screening of Ethanolic extract of Neem leaf extract

Solvent	**Phytochemicals**						
Ethanol	**Alkaloid**	**Flavonoid**	**Terpenoid**	**Glycoside**	**Phenol**	**Tannins**	**saponnins**
	++	+	+	-	-	-	+++

Note + represents the presents of the constituents
- represents the absent of the constituents

Table 2: Physicochemical Parameters of the Ethanolic Extract of Neem leaf extract

Solvent	Physicochemical Parameters		
Ethanol	**Iodine value**	**pH**	**Conductivity**
	0.634	6.42	48.2

Table 3: Antibacterial activity of Neem ethanolic leaves extracts against the tests organisms

Test organisms	Concentration (mg/ml)				Control (Cefriaxone)			
	2	4	6	8	2	4	6	8
	Zone of inhibition in (mm)				Zone of inhibition in mm			
Escherichia coli	0.00	0.00	0.00	0.25	0.48	1.92	2.88	3.84
Salmonella spp	0.00	0.00	0.28	1.03	1.63	6.50	9.72	13.00

4.2 Discussion

Medicinal herbs constitute effective sources of antimicrobial and antioxidant natural products (Calixto, 2000). Medicinal herbs are important sources for the therapeutic remedies of various ailments (Moses and Maobe, 2013). In this present study, the phytochemical (Qualitative analysis) of neem ethanolic leaf extract revealed the presence of alkaloids, flavonoids, terpenoids and saponin. This is in agreement with the work done by (Krishnaveni *et al.,* 2014; Zahir *et al.,* 2014) both in India. Another study by Alli *et al.*, (2011) in Kano State Nigeria revealed the presence of all the parameters in *Azadirachta indica*. These classes of compounds especially flavonoids, alkaloids and saponins, are known to have curative activity against several pathogens (Usman *et al.,* 2009). The presence of alkaloids in neem leaf extract in this study shows the potential of the extract to have an analgesic, anti-inflammatory and adaptogenic effects, which help the host (man and animal) to develop resistance against disease and endurance against stress (Gupta, 1994). Similarly, saponins are steroid or triterpenoid glycosides characterized by their bitter or astringent taste, foaming properties and their haemolytic effect on red blood cells. Saponins possess both beneficial (cholesterol-lowering) and deleterious (cytotoxic permeabilization of the intestine) properties and also exhibit structure dependent biological activities (Osagie and Eka, 1998). Saponins cause a reduction of blood cholesterol by preventing its reabsorption (Prohp and Onoagbe, 2012). More so, Glycosides have a strong and direct action on the heart, helps in supporting its strength and rate of contraction when it is failing (Persinos and Quimby, 1967).

The results obtained from this study shows clearly the various physicochemical parameters of extract examined. The pH ranged obtained from this study was 6.42 for the sample. This falls within the Standard Organization of Nigeria (SON) and by World Health Organization (WHO) limits (SON, 2007; WHO, 2011). It should be noted that high pH increases the toxicity of ammonia in water while low pH enhances the toxicity of H_2S and cyanide (Ibiebele *et al.,* 1983). Since the pH values of this extract is basic, they sample may pose no serious health risk to consumers who use the extract for treatment of ailments and other traditional purposes. The conductivity value was 48.2 although these values fell within the acceptable limits. The overall chemical richness of any extracts is a reflection of its conductivity values. The relatively low conductivity values may be attributed to low concentrations of chloridet. The conductivity of the plant extracts is a useful and accessible indicator of its salinity or total salt content (Oluyemi *et al.,* 2010).

The results for the antibacterial screening have revealed that though at varying concentrations, neem extracts have antibacterial activity on the *Salmonella spp* and *Escherichia coli*. However, the results generated from the study indicate that the ethanolic extract of neem leaf has antibacterial activity with variable degree of sensitivity. In addition, it was observed that the more the concentration the more the zone of inhibition. This research is in agreement with the research reported by Naser *et al*. (2012) in Saudi Arabia who reported the effects of crude extracts of neem on bacteria isolates showing higher inhibition of 8.00 and 16.0mm on *Escherichia coli* and *Streptococcus* spp respectively. The results showed that zone size increases with increase in concentration of the extracts.

Herbal plants might have a great potential to be used as antimicrobial agents by Zaika (1988) has given an excellent summary of the antimicrobial effectiveness of herbs. This summary can be rephrased as follows; microorganisms differ in their resistance to a given herbs, bacteria are more resistant than fungi, spores might be more affected, Gram-negatives are more resistant, herbs effects could be germicide or germistatic, they may contain microbes and promote growth and toxins, their concentrations added as food preservatives are too low in order to be preventive, and other factors; e.g. NaCl may contribute synergistically. Chemical and biochemical antimicrobial compounds derived from natural sources and their activity against a range of pathogenic and spoilage microorganisms pertinent to food, together with their effects on food organoleptic properties, are outlined. Aqueous extracts showed

more antibacterial activities compared to methanolic extract and factors influencing the antimicrobial activity of such agents might be attributed to extraction methods, molecular weight, and agent origin. These issues are considered in conjunction with the latest developments in the quantification of the minimum inhibitory (and non-inhibitory) concentration of antimicrobials and/or their components. Natural antimicrobials can be used alone or in combination with other novel preservation technologies to facilitate the replacement of traditional approaches (Tiwari *et al.,* 2009). Thus, food product safety and shelf life depend in some part on the type, quantity, and character of herbs added to the products.

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

In this study, neem ethanolic extracts exhibited varying levels of antibacterial activity against *Salmonella spp* and *Escherichia coli.* The antimicrobial activity of neem extract varies widely, depending on the type of herb on the test medium, and microorganism. For these reasons, neem leaf might not be considered as excellent antimicrobials agents.

5.2 Recommendations

From the results obtained in this study, it is recommended that:

1. Experiments should be carried out at higher concentrations of the aqueous and methanolic extracts to assess their activity on multidrugs resistant organisms (MRSA and PRSA)
2. Research should be carried out using bioassay guided fractionation to identify, isolate and characterize the bioactive components of the plant extracts.
3. The ministry of health should make it mandatory that all herbal products be subjected to scientific verifications before being sold as remedy.

ACKNOWLEDGMENTS

This research was funded by TETFUND under the Institution Based Research (IBR) Annual Intervention, we therefore acknowledged their immense support. Also appreciates Federal College of Education Gidan Madi, Umaru Ali Shinkafi Polytechnic Sokoto for the Laboratory space to conduct this work.

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