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ANTIMICROBIAL PROPERTIES OF TRACE PHYTOCHEMICAL COMPOUNDS ANALYSIS OF *Azadirachta indica* LEAVES IN *Acinetobacter baumannii* ISOLATED FROM SPUTUM SPECIMEN

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ABSTRACT

Antimicrobial properties of trace phytochemical compounds analysis of *azadirachta indica* leaves in *acinetobacter baumannii* isolated from sputum specimen. *Azadirachta indica* L. leaves was collected from Imo State University Farm, Owerri, Imo State. The aqueous and ethanolic extracts of the plants was extracted from 100g of dried powdered leaves with 100ml sterile distilled water and 100ml ethanol respectively using Soxhlet method. Fifty (50) sputum specimens was collected from patients admitted at Federal Medical Center Owerri for the isolation of *Acinetobacter baumannii.* Identification of *Acinetobacter baumannii* was done using morphological characteristics and biochemical test. Antimicrobial analysis of the characterized phytochemical compounds of *Azadirachta indica* leaves was tested against *Acinetobacter baumannii* using agar well diffusion. Two milliliters of each extract concentration was gently be mixed with the Mueller Hinton Agar and poured into Petri dishes to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts. Results shows Minimum Inhibitory Concentrations and Minimum Bactriocidal Concentration of A*zadirachta indica* extract against *A. Baumanni* showed No growth for at 25%, 50% and 100%. Qualitative phytochemical analysis on acetone and aqueous of *Azadirachta indica* leaves extracts showed the presence of Saponins, Flavonoid, Phenol, Alkaloid and Tannin. Antimicrobial effect of aqueous extract and ethanol of the A*zadirachta indica* leaves on extract on A. *Baumannii* showed no growth for 20%, 50% and 100% respectively.This study revealed the effects of aqueous extracts of *Azadirachta indica* leaf on *Acinetobacter baumannii*, as offer bacteria an attractive environment in which they can potentially flourish whereby causing significant damage if left untreated. While ethanol extract showed slightly turbid with growth.

INTRODUCTION

Over the years, medicines and medicinal agents derived from plants have made large contributions to human health and well-being. This is because they are either used directly as phytomedicines for the treatment of various ailments or they may become the base and the natural blueprint for the development of new drugs (Cseke *et al.*, 2016).

Herbal medicine also called phytotherapy or phytomedicine has been around since the beginning of recorded history. It has also been described as the therapeutic use of medicinal plants referred to as herbs (Thea *et al.,* 2008). Herbal medicine has become an integral part of standard health care, based on a combination of time honored traditional usage and ongoing scientific research. Surging interest in medicinal herbs has increased scientific scrutiny of their therapeutic potential and safety. Some of the medicinal plants are believed to enhance the natural resistance of the body to infections (Atal *et al.,* 2016).

According to the World Health Organization (WHO), herbal medicines could also be referred to as phytopharmaceuticals sold as over the counter products in modern dosage forms such as tablets, capsules, syrups or liquids for oral use or dietary supplements containing herbal products, also called nutraceuticals available in modern dosage forms, or even referred to as medicines consisting of other crude, semi processed or processed medicines, which have a vital place in primary health care and developing countries like Nigeria.

Dogoyaro also known as Neem plants (*Azadirachta indica*) are mostly trees and rarely shrubs that belong to family Maliacea (Margaret, 2015). The plant has been used for a long time in agriculture and medicine (Natarajan *et al*., 2013). Dogoyaro is a widely distributed Nigeria indigenous plant. People from different countries have also learnt about this miraculous plant, carried seeds with them to grow in their respective countries (Bodurin, 2009). The importance of the dogoyaro tree has been recognized by US National Academy of Sciences, which published a report in 1992 entitled " Dogoyaro -a tree for solving global problems" (Biswas *et al.*, 2012). It is established that many scientific studies that dogoyaro seeds contain chemical compounds to control more than 100 species of insects

and microorganisms (Vaideki *et al*., 2007). Every part of the tree has been used as traditional medicine for household remedy against various human ailments, from antiquity (Thakur *et al*., 2011). The excellent bioactivity of this dogoyaro products are attributed to the chemical compounds such as nimbin, nimbidin and salanin (Rao *et al*., 2016). Dogoyaro leaves are reported as the main sources of the active compounds obtained from the plant. Dogoyaro leaf contains several valuable components such as isoprenoids that include terpenoids containing limonoids, azadirone and its derivatives (Xu *et al*., 2010).

The medicinal properties of the plant were studied by several workers. The antipyretic effect (Kirtikar and Basu, 2015) anti-malaria effect, anti-diabetic effect (Shukla and Bhandari, 2013), antifertility effect (Sinha *et al*., 2014), effect on the central nervous system, cardiovascular effect (Thompson and Anderson, 2008) and wound healing (Jayaprakasan *et al*., 2014) were some of the studies of workers. *A. indica* has been shown to possess anti-microbial properties by several studies. Rao *et al*. (2016) reported the antimicrobial activity of the seed oil against a variety of pathogens. Oils from the leaves, seeds and bark possess antibacterial action against certain bacteria (Khan and Wassilar, 2017). Extracts of dogoyaro leaf, dogoyaro oil and seed kernels are effective against certain human fungi (Biswas *et al.,* 2012). More than 135 compounds have been isolated from different parts of neem and several reviews have also been published on the chemistry and structural diversity of these compounds (Khan and Wassilar, 2017).

MATERIALS AND METHODS

SAMPLE COLLECTION

COLLECTION OF *Azadirachta indica* L.

Azadirachta indica L. leaves was collected from Imo State University Farm, Owerri, Imo State and was identified in the Department of Botany before being transported to the Department of Microbiology, Imo State University for extraction and antimicrobial analysis.

COLLECTION OF SPUTUM SPECIMEN

Fifty (50) sputum specimens was collected from patients admitted at Federal Medical Center Owerri for the isolation of

Acinetobacter baumannii. Patients was asked to take a very deep breath and hold the air for 5 seconds. Slowly breathe out. Take another deep breath and cough hard until some sputum comes up into their mouth before spitting the sputum into the sterile universal container. The sputum specimen was transported in a sterile poly ethylene bag to the Department of Microbiology, Imo State University Owerri for immediate analysis.

ISOLATION AND CHARACTERIZATION OF *Acinetobacter baumannii*

Isolation and characterization of *Acinetobacter baumannii* was carried out using standard microbiological techniques as described by Cheesbrough (2002). Prepared Chromo *Acinetobacter* agar was aseptically dispensed into sterile petri dish and was allowed solidify. With the help of a sterile wire loop, sputum specimen was used to streak on the surface the dried agar Chromo *Acinetobacter* agar. The plates was labeled at the bottom with the sample code, date and time. The plates was incubated aerobically at 37^0 C for 24 - 48 hours. At the end of incubation, *Acinetobacter baumannii* isolates supplemented was appear red and other *Acinetobacter* species was appear blue. Pure colonies was kept in nutrient agar slants. The nutrient agar slants was incubated at 37^0 C for 18 – 24 h before storage in the refrigerator at 4^0 C pending biochemical analyses.

PREPARATION OF *Azadirachta indica* FOR EXTRACTION

After collection, the leaves of *Azadirachta indica* was washed using tap water from the laboratory and was air dried at room temperature for 10 days and then was grounded into fine powder using a blender and will be kept in the refrigerator prior to use. The aqueous and ethanolic extracts of the plants was extracted from 100g of dried powdered leaves with 100ml sterile distilled water and 100ml ethanol respectively using Soxhlet method. The extracts of the plant was evaporated in an oven at 40^OC, and then was used for phytochemical screening and microbiological studies (Nwachukwu *et al.,* 2006).

ANTIMICROBIAL ANALYSIS OF CHARACTERIZED PHYTOCHEMICAL COMPOUNDS OF *Azadirachta indica* LEAVES

Antimicrobial analysis of the characterized phytochemical compounds of *Azadirachta indica* leaves was tested against *Acinetobacter baumannii* using agar well diffusion method as described by Adeniyi *et al.* (2016) was adopted and modified slightly. A 0.5 McFarland standard equivalent suspension of *Acinetobacter baumannii* was made in 0.85 % normal saline and 0.1 mL of it was used to inoculate plates of Mueller Hinton Agar. Equidistant wells was bored with the aid of a standard sterile 8mm cork borer and 0.1 ml of different concentrations of ethanol extract and controls was placed into the corresponding wells. Ciprofloxacin (5 µg/ml) was used as the standard drug. The screening was done in duplicates. The plates was allowed to stay at room temperature for an hour to allow for pre-diffusion of the extracts into the agar medium. The plates was then be incubated at 37 °C for 24 hours. The same procedure was repeated using water and methanol extracts of the plant.

DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC) AND MINIMUM BACTERICIDAL CONCENTRATION (MBC) OF THE EXTRACTS

Eighteen milliliters of Mueller Hinton Agar (MHA) was prepared and sterilized in McCartney bottles. Two milliliters of each extract concentration was gently be mixed with the Mueller Hinton Agar and poured into Petri dishes. Thereafter, a 0.5 McFarland standard equivalent suspension of each isolate was made in 0.85 % normal saline from which 0.1 mL was used to inoculate the plates. The plates was then be incubated at 37 °C for 24 hrs. The MIC was recorded as the lowest concentration of the extract that was inhibited the growth of the test organisms (Adeniyi *et al.,* 2016).

RESULT

Table 1 shows the results of the Biochemical characterization of *A. baumannii* isolates from wound specimen.

TABLE 1: PHYTOCHEMICAL SCREENING OF NEEM (*Azadirachta indica*) EXTRACT

Phytochemical Components	**Extracts**
	Aqueous
Alkaloids	++
Saponins	+
Tannins	+
Flavonoid	++

Key: +++ high amount; ++ moderate amount; + trace/minute amount

Qualitative phytochemical analysis on acetone and aqueous of *Azadirachta indica* leaves extracts showed the presence of Saponins, Flavonoid, Phenol, Alkaloid and Tannin (Table 2).

TABLE 2: MIC AND MBC OF NEEM (*Azadirachta indica*) EXTRACT AGAINST A. BAUMANNII

Microorganism	**25%**	**50%**	**100%**	**MIC%**	**MBC%**
A. baumannii	-	-	-	<100	-

Key : - No growth, ++ = very turbid with growth, + = Slightly turbid with growth

MIC = Minimum Inhibitory Concentrations

MBC = Minimum Bactriocidal Concentration

TABLE 3: BIOCHEMICAL CHARACTERIZATION OF *A. baumannii* OBTAINED FROM SPUTUM SPECIMENS

Isolate	Gram reaction	Motility	Oxidase	Catalase	Indole	Methyl Red	Glucose
A. baumannii	-	-	-	+	-	-	+

TABLE 4: ANTIMICROBIAL EFFECT OF AQUEOUS EXTRACT OF THE NEEM (*Azadirachta indica*) leaves ON EXTRACT ON *A. baumannii*

Concentration of Extracts in (%)	Zones of inhibitions (mm)
	A. baumannii
25%	-
50%	-
100%	-

TABLE 5: ANTIMICROBIAL EFFECT OF ETHANOLIC EXTRACT OF THE NEEM (*Azadirachta indica*) leaves ON EXTRACT ON *A. baumannii*

Concentration of Extracts in (%)	Zones of inhibitions (mm)
	A. baumannii
25%	-
50%	-
100%	+

DISCUSSION, AND CONCLUSION

This study revealed the effects of aqueous extracts of *Azadirachta indica* leaf on *Acinetobacter baumannii*, as offer bacteria an attractive environment in which they can potentially flourish whereby causing significant damage if left untreated (Table 1). In the serial exhaustive (sequential) extraction of the test plant extracted with water. Therefore, the amount of yield extracted is high in polar solvents than the non-polar solvents. The polarity of the different solvents may be responsible for variations in solubility of active phyto-components of the plant, hence, difference in efficacy levels (Ahmed *et al.,* 2017). Method of extraction is important in antimicrobial investigations as this determines to a large extent the outcome of the study (Anyanwu and Okoye, 2017). The solvents of choice in this study of sequential extraction system for the extraction of *Azadirachta indica* leaves are water. Thereafter, the extracts were made to undergo sterility test and all proved sterile as there was no growth observed after incubating for 24 hours. Phytochemical screening of the aqueous extract of *Azadirachta indica* as shown in Table 2 revealed alkaloids, saponins, tannins and flavonoids to be present in the plant extracts. In aqueous extract of the plant, it was observed that saponins and tannins were in high quantity while alkaloids and flavonoids were in moderate amount. These results obtained are in general agreement with the reports of Raimi *et al.,* (2014). The presence of these active components in studied part of the plants may be responsible for its antimicrobial sensitivity on the test organisms as reported by some scientists (Kolapo *et al.,* 2009; Zakariyah *et al.,* 2017). The antimicrobial sensitivity of the test organisms to aqueous extract of *Azadirachta indica* leaf is shown in Tables 4. Antimicrobial activities of n-hexane extract of *Azadirachta indica* leaf wasn't significant against the test organisms, this might be due to the method of extraction and the morphological characteristics of the test isolates as observed in the results which is contrast from Ayanwale *et al.,* (2019). This work is in contrast with the result reported by *Ghaddar et al.,* (2020). The wide range of activity shown by the samples in this study appears to provide an acceptable explanation

of the scientific basis for their uses in traditional medicine. It is hoped that this study would lead to further investigations that would enhance the preparation of antimicrobial drugs of natural origin for the treatment of sputum caused by the test isolates.

CONCLUSION

This study has revealed that the leaves of *Azadirachta indica* have chemicals (compounds) of intermediate polarity and possess potentially active antimicrobial agents that are inhibitory to the test organisms. No significant activity of the plant extracts against *Acinetobacter baumannii* confirms its further investigation. Also, it was revealed in this study that the solvents used for extraction plays a vital role in the level of activity displayed by the plant.

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