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PHYTOCHEMICAL AND ANTIMICROBIAL STUDIES OF ROOT BARK EXTRACTS FROM *GLOSSONEMA BOVEANUM (*DECNE)

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ABSTRACT

The root bark of *Glossonema boveanum* (Decne), a member of *Apocynaceae* family, is use by traditional medicine practitioner to treat urinary and respiratory tract infections, bacteremia, typhoid fever, bacillary dysentery, diarrhoea and stomach pain. This present study is aim to validate the medicinal claims ascribed to the root bark of the plant. Preliminary phytochemical study of the root bark extracts (n-hexane, ethyl acetate, chloroform and methanol extracts) showed the presence of alkaloids, carbohydrates, steroids, triterpenes, cardiac glycosides, saponins, tannins and flavonoids. Antimicrobial study of the extracts showed activities against *Staphylococus aureus, Bacillus subtilis, Salmonella typhii, Shigella dysenteriae, Escherichia coli, Enterobacter cloacae*, *Streptococcus agalactiae* and *Candida albicans* while *Micrococcus luteus, Pseudomonas aeruginosa* and *Klebsiella Pneumoniae* showed resistance to all the extracts. The inhibitory effect was compared with the standard drug ciprofloxacin and fluconazole. MIC and MBC for both extracts were also determined using the tube dilution method. This study concluded that the root bark of *G. boveanum*, used traditionally as a medicinal plant, has antimicrobial activities against some causative organisms.

KEYWORDS: ***Glossonema boveanum* (Decne.), phytochemical, antimicrobial, minimum inhibitory concentration, minimum bactericidal concentration**

INTRODUCTION

According to World Health Organization, a medicinal plant is defined as any plant in which, one or more of its organs contain substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs [1]. Since ancient times, natural products obtained from plant sources remain a major source of preventive and curative medicine. A large number of human populations in developing countries still depend on the medicinal properties of plants for their preventive and curative properties [2].

Medicinal plant researchers explore several goals like the development of low cost therapeutic compounds and the discovery of prototypic drugs [3]. These bioactive constituents from plant origin show antimicrobial activity against some microorganisms like bacteria, fungi and protozoa [4].

The medicinal plant *Glossonema boveanum* (*Decne*) belongs to the family *Apocynaceae* and is commonly known as *Taarin gida* (Hausa), *Achakan* (Arabic), *Gobble daurobe* (Fula-fulfulde) [5]. *G. boveanum* is widely distributed in African countries and exists as perennial plant [6]. It is a hairy canescent herb that reaches up to 10 – 30 cm long, its stems are much-branched from the base, and the leaves are 1-2 × 0.5-1.2 cm ovate in shaped. Its flowers are whitish-yellow corolla, and the fruit is a cylindrical or follicle with flattened, ovate, brown seeds [7, 8].

G. boveanum is commonly used locally for treating urinary and respiratory tract infections, bacteremia, typhoid fever, bacillary dysentery, diarrhea and stomach pain, as well as increasing lactation in breastfeeding women [5] [9].

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There have been reports from literature on the phytochemical and antibacterial screening of stem bark extract from *Glossonema boveanum* [10], Isolation of flavonoid glycosides, alpha-amyrin acetate, lupeol and betulinic acid from the aerial part of the plant [11-13].
However there is dearth of information regarding the phytochemical study, antimicrobial activity, and isolation of a compounds from the root bark of *G. boveanum*, and hence the need to validate the medicinal claims ascribed to the root bark of this plant becomes necessary.

Figure 1.0: A cross section of *Glossonema boveanum* plant showing the leave, fruits and stem

MATERIALS AND METHODS

Plant Material

The plant material was collected from the wild, Garin Lamido, Gashua, Yobe state, Nigeria. It was authenticated at the herbarium section of the Department of Biological Sciences, Ahmadu Bello University, Zaria – Nigeria. A voucher specimen number: 4487 was assigned and the specimen deposited at the herbarium for reference purpose. The root bark was air-dried for 21 days and then crushed to coarse powder.

Extraction of the Root Bark

The pulverized plant sample (1000 g) was macerated successively in n-hexane, ethyl acetate, chloroform and methanol exhaustively until complete extraction.

Phytochemical Screening

Preliminary phytochemical screening of the plant extracts was carried out using standard method as described by [14].

Microorganisms

The microorganisms used include *Staphylococus aureus, Bacillus subtilis, Salmonella typhii, Shigella dysenteriae, Escherichia coli, Enterobacter cloacae*, *Streptococcus agalactiae, Candida albicans, Micrococcus luteus, Pseudomonas aeruginosa* and *Klebsiella Pneumoniae.* They were obtained from the Department of Microbiology, Nigeria Institute of Leather and Science Technology, Zaria - Nigeria. All the isolates were checked for purity and maintained in a slant of Nutrient agar.

Sensitivity Test

The agar well diffusion method was used [15] [16]. The antimicrobial activities of the n-hexane, ethyl acetate and methanol extracts of the root bark of *Glossonema boveanum* were determined using stock concentration of 100 mg/mL. Standardized inocula of the isolates were uniformly streaked unto freshly prepared Mueller Hinton Agar plates with the aid of a sterile swab stick. Using a sterile cork borer (3 mm diameter), one well was punched into the agar plate. Thereafter, 0.2 mL of the appropriate extract concentration was placed in the well and allowed to diffuse into the agar. An extra plate was also streaked with the inocula and ciprofloxacin standard (10mg/ml) was placed on it. The plates were incubated at 37˚C for 24 hours. While for the fungus, Sabouraud dextrose agar was used at an incubation period of 72 hours at 25˚C. The antimicrobial activities were expressed as diameter zones of inhibition produced by the plant extracts and reported in millimeter (mm). Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) for both extracts were also determined using the tube dilution method.

RESULTS AND DISCUSSION

This study shows that the root bark extracts (n-hexane, ethyl acetate, chloroform and methanol extracts) exhibited good antimicrobial activity against the tested organisms (Table 4.2). The inhibitory effect was compared with the standard drug ciprofloxacin and fluconazole. The Minimum inhibitory concentration and Minimum bactericidal concentration of the extracts were also determined and were respectively presented in table 4.3 and 4.4. These inhibitory activities of the extracts confirmed the potential use of the plant in the treatments of microbial induced ailments.

Table 4.1: Phytochemical constituents of the root bark of *G. boveanum*

The table shows the phytochemical constituents of n-hexane extract, Ethyl acetate extract, chroroform extract and methanol extract from the root bark of *Glossonema boveanum*.

Phytochemical	Test	n-Hexane	Ethyl acetate	Chloroform	Methanol
Alkaloids	Dragendorff	–	–	–	+
	Mayer	–	–	–	+
Carbohydrates	Molisch	+	+	+	+
	Fehling	+	+	+	+
Anthraquinones	Bontrager	–	–	–	–
Steroids	Liebermann-Buchard	+	+	+	+
Triterpenes	Liebermann Buchard	+	+	+	+
Cardiac Glycosides	Killer-killiani	+	+	+	+
Saponins	Frothing	–	+	–	+
Tannins	Ferric chloride	–	+	+	+
Flavonoids	NaOH	–	+	+	+
	Shinoda	–	+	+	+

+ Presence; – Absence

Table 4.2: Antimicrobial Activity of Root Bark Extracts of *G. boveanum*

The table shows the zone of inhibition (mm) of n-hexane extract, Ethyl acetate extract, chroroform extract and methanol extract from the root bark of *Glossonema boveanum.* The table also shows the zone of inhibition (mm) of Ciprofloxacin and Fluconazole.

Test Organisms	Zone of Inhibition (mm)				Ciprofloxacin 10 mg/ml	Fluconazole 10 mg/ml
	Meth.	Eth.	Chlo.	Hex.		
Staphylococcus aureus	22	25	27	25	35	0
Bacillus subtilis	23	29	28	27	37	0
Klebsiella Pneumoniae	0	0	0	0	30	0
Streptococcus agalactiae	21	30	25	28	32	0
Salmonella typhii	20	29	28	25	41	0
Shigella dysenteriae	20	22	25	23	40	0
Micrococcus luteus	0	0	0	0	0	0
Candida albicans	22	28	24	26	0	34
Escherichia coli	22	30	26	28	39	0
Enterobacter cloacae	24	31	27	25	35	0
Pseudomonas aeruginosa	0	0	0	0	0	0

Meth. = Methanol, Eth. = Ethyl acetate, Chlo. = Chloroform, Hex. = n-Hexane

Table 4.3: Minimum Inhibitory Concentration (MIC)

The table shows the minimum concentration of n-hexane extract, Ethyl acetate extract, chroroform extract and methanol extract that will inhibit the growth of the tested microorganism.

Test organisms	Extracts (mg/mL)			
	Methanol	Ethyl acetate	Chloroform	n-Hexane
Staphylococcus aureus	1.00	0.250	0.250	0.250
Bacillus subtilis	1.00	0.125	0.125	0.125
Klebsiella Pneumoniae	–	–	–	–
Streptococcus agalactiae	1.00	0.125	0.125	0.125
Salmonella typhii	1.00	0.125	0.250	0.250
Shigella dysenteriae	1.00	0.125	0.250	0.250
Micrococcus luteus	–	–	–	–
Candida albicans	1.00	0.125	0.250	0.250
Escherichia coli	1.00	0.125	0.125	0.125
Enterobacter cloacae	1.00	0.125	0.250	0.250
Pseudomonas aeruginosa	–	–	–	–

Table 4.4: Minimum Bactericidal Concentration (MBC)

The table shows the minimum concentration of n-hexane extract, Ethyl acetate extract, chroroform extract and methanol extract that will completely kill the tested microorganism

Test organisms	Extracts (mg/mL)			
	Methanol	Ethyl acetate	Chloroform	n-Hexane
Staphylococcus aureus	4.00	0.500	0.500	0.500
Bacillus subtilis	2.00	0.250	0.500	0.500
Klebsiella Pneumoniae	–	–	–	–
Streptococcus agalactiae	4.00	0.250	0.250	0.250
Salmonella typhii	4.00	0.250	0.500	0.500
Shigella dysenteriae	2.00	0.250	0.250	0.250
Micrococcus luteus	–	–	–	–
Candida albicans	4.00	0.250	0.500	0.500
Escherichia coli	4.00	0.250	0.250	0.250
Enterobacter cloacae	2.00	0.250	0.500	0.500
Pseudomonas aeruginosa	–	–	–	–

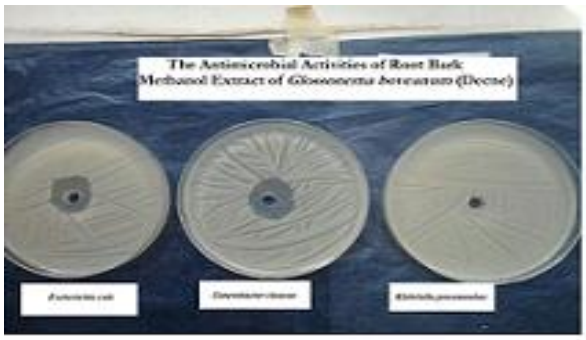

Plate 4.1 Antimicrobial Screening of Methanol extract

Plate 4.2 Antimicrobial Screening of Methanol extract

Plate 4.3 Antimicrobial Screening of Methanol extract

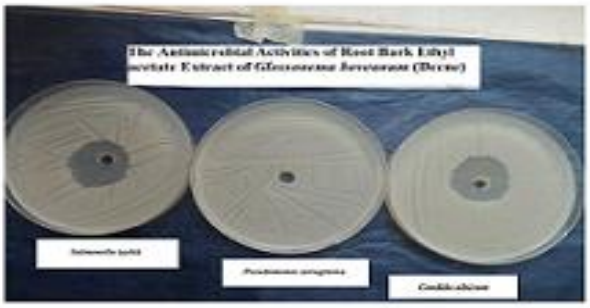

Plate 4.4 Antimicrobial Screening of Ethyl Acetate extract

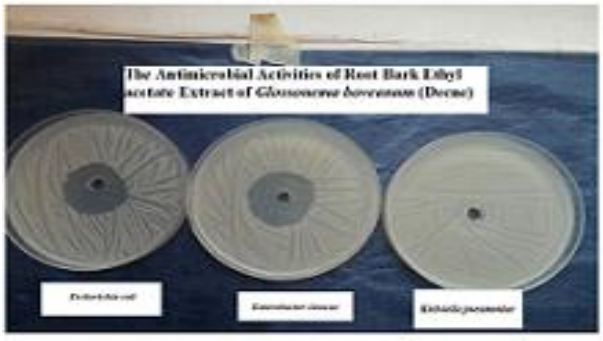

Plate 4.5 Antimicrobial Screening of Ethyl Acetate extract

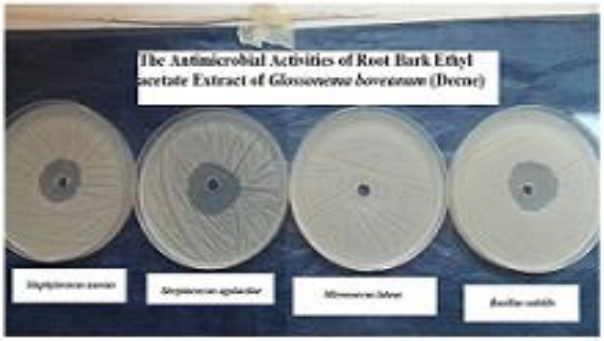

Plate 4.6 Antimicrobial Screening of Ethyl Acetate extract

Plate 4.7 Antimicrobial Screening of Chloroform extract

Plate 4.8 Antimicrobial Screening of Chloroform extract

Plate 4.9 Antimicrobial Screening of Chloroform extract

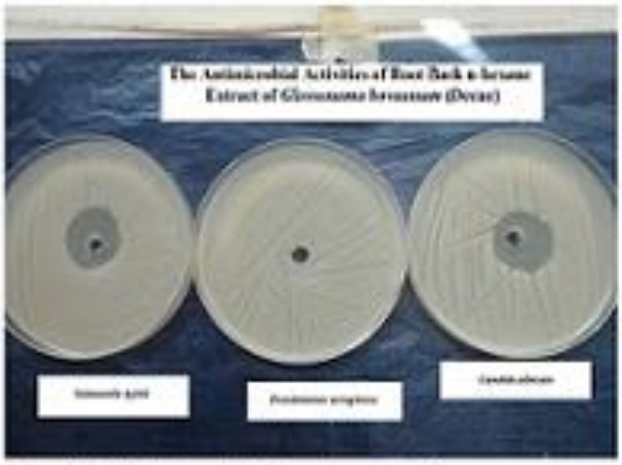

Plate 4.10 Antimicrobial Screening of n-Hexane extract

Plate 4.11 Antimicrobial Screening of n-Hexane extract

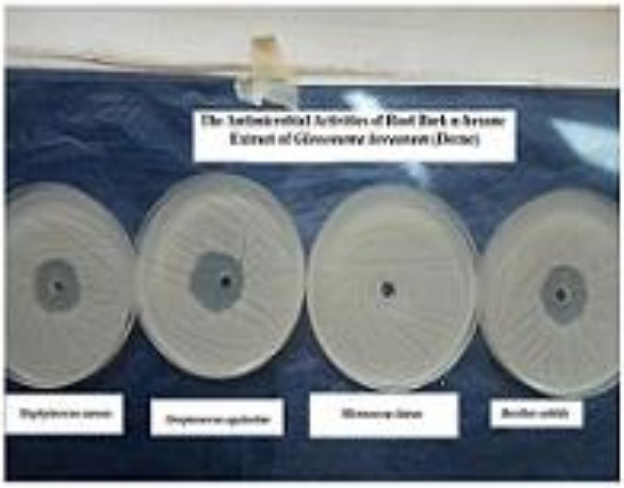

Plate 4.12 Antimicrobial Screening of n-Hexane extract

As reported in table 4.1, the phytochemical study shows the presence of some phytoconstituents. The result indicates that methanol and ethyl acetate are more efficient solvents for extraction of the phytoconstituents from the root bark of *G. boveanum*. These phytochemicals might be

responsible for the observed good antimicrobial activities and can be seen as a potential source of useful drugs. In general, the accumulation and concentration of secondary metabolites are responsible for antibacterial activity and this varies according to plant extracts depending on their polarity [17].

Flavonoids possess antibacterial, antifungal and antiviral activity [18]. Tannins are known for their astringent property and antimicrobial activity [19]. Alkaloids have served as scaffolds for important antibacterial drugs [20]. Saponins possess antibacterial and anticandidal [21].

The extracts showed significant activities against *E. cloacae* the bacteria responsible for bacteremia, lower urinary and respiratory tract infections. The extracts also showed significant activities against *E. coli*, the bacteria responsible for diarrhea and stomach pain. The sensitivity of *S. typhii, S. aureus*, *B. subtilis*, *S. agalactiae* to all the extracts implies that chemical compounds in the extracts could be used to develop drugs to treat related ailments. The use of the plant for the treatment of urinary and respiratory tract infections, typhoid fever, bacteremia, diarrhoea and stomach pain is justified since these microbes are responsible for such illness [22]. The extracts also showed good activities against *S. dysenteriae*, the bacteria responsible for bacillary dysentery. Therefore, all the extracts could serve in one way or the other as source of compounds that may be effective in the management of the ailments associated with the causative agents.

CONCLUSION

The present study on phytochemical investigation of root bark from *Glossonema boveanum* reveals the presence of various phytochemical constituents which support its use in local medicine. This study also helps us to carry out researches and to confirm scientifically the antimicrobial activities of *G. boveanum*. Our study suggests that the root bark of *G. boveanum* may be a potential source for antimicrobial drug discovery.

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CONFLICT OF INTEREST

Authors declared no conflict of interests in te conduct and reporting of this study

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