Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2015; 4(2): 86-88
Received: 05-05-2015
Accepted: 07-06-2015

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Phytochemical and Antimicrobial Screening of Stem Bark Extracts from *Glossonema boveanum (*Decne)

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Abstract
The medicinal plant *Glossonema boveanum* (Decne) is known locally for its medicinal value. Preliminary phytochemical screening of the extracts from stem bark using standard procedures showed positive test for alkaloids, carbohydrates, steroids, triterpenes, cardiac glycosides, saponins, tannins and flavonoids. The antimicrobial activity against *Staphylococus aureus, Bacillus subtilis, Streptococcus agalactiae, Shigella dysenteriae, Salmonella typhii, Escherichia coli,* and *Candida albicans* was carried out on methanol, ethyl acetate and n-hexane extracts using Agar well diffusion method and broth dilution method. The extracts were found to be effective against all the pathogens except *S. agalactiae* and *C. albicans*. Minimum Inhibitory Concentration (MIC) results revealed that Ethyl acetate and Methanol extracts inhibits the growth of the tested microorganisms and was as low as 3.125µg/mL**.** Our findings showed that the extracts from the stem bark of *G. boveanum* contain potential antimicrobial compounds worthy of further investigation. This claim supports the ethno medicinal usage of the plant.

Keywords: *Glossonema boveanum* (Decne.), phytochemical screening, antimicrobial activity, minimum inhibitory concentration.

1. Introduction
Plant-derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are the richest bio logical resources of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs [1]. Since ancient times, natural products obtained from plant sources remains as a major source of preventive and curative items. This result in the large number of human population still being dependent on the medicinal plants for their preventive and curative properties. According to World Health Organization (2011), traditional medicines, including herbal medicine, have been, and continue to be, used in every country around the world in some capacity. In much of the developing world, 70-95% of the population relies on these traditional medicines for primary healthcare [2].

Herbal formulations have reached widespread acceptability as therapeutic agents for diabetics, arthritis, liver disease, cough remedies and memory enhancers. According to WHO definition, there are three kinds of herbal medicines: raw plant material, processed plant material and medicinal herbal products [3]. Herbal medicines are widely used in the healthcare sector in both developed and developing countries. These are complex chemical mixtures prepared from plants and are limited in their effectiveness because they are poorly absorbed when taken orally [4].

As a result of indiscriminate use of antimicrobial drugs in the treatment of infectious diseases, microorganisms have developed resistance to many antibiotics. There is therefore the need to develop alternative antibiotic drugs from plants. One approach is to screen local medicinal plants which represent rich source of novel antimicrobial agents.

Glossonema species belong to the family Apocynaceae and are used in traditional medicine to cure various ailments. They are widely distributed in African countries, and exist as perennial plants [5, 6]. Antifungal and antibacterial activities have been reported from hexane, ethyl acetate and methanol extracts of some species from Apocynaceae [7, 8].

Glossonema boveanum, has been used in traditional medicine for the treatment of several diseases such as Pelvic inflammatory disease, Gonorrhoea, Chlamydia, Haemorrhoid, etc. This paper reports the phytochemical constituents present in extracts from *G. boveanum* and its antimicrobial activity against some pathogens.

2. Materials and Methods

2.1. Plant Material

Fresh leaves, stems and root of *Glossonem boveanum* were collected from Utupko village, Benue State in the month of January, 2014. The leaves were identified and authenticated by Mallam Musa Mohammed of Herbarium section of Biological Science Department, Ahmadu Bello Univeristy Zaria – Nigeria. Voucher number was (4487) was deposited there for further reference.

2.2. Extraction of the Stem Bark

The stem bark was removed from the stem, air dried and crushed to coarse powder. The powdered stem bark (1000g) was successively extracted with hexane, ethyl acetate and methanol using cold maceration till exhaustion. All the extracts were evaporated in a rotary evaporator at 25 ^{0}C under reduced pressure [9].

2.3. Phytochemical Screening

Preliminary phytochemical screening of the plant extracts was carried out using standard method as described by [10].

2.4. Microorganisms

The microorganisms used include *Staphylococus aureus, Bacillus subtilis, Streptococcus agalactiae, Salmonella typhii, Shigella dysenteriae, Escherichia coli* and *Candida albicans.* They were obtained from the Department of Microbiology, Ahmadu Bello University Zaria - Nigeria.

2.5. Anti Microbial Activity

Sensitivity test: The agar well diffusion method was used [11]. The antimicrobial activities of the n-hexane, ethyl acetate and methanol extract of the stem bark extracts bark of *Glossonema boveanum* were determined using stock concentrations of 100 mg/ml. The 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 (8 mm in diameter), five appropriately labeled wells were punched into each agar plate. 0.2 ml of the appropriate extract concentration was placed in each well and then allowed to diffuse into the agar. The plates were incubated at 37 ^{0}C for 24hours. However Sabouraud dextrox agar was used for the fungi and the incubation period was 48hours [12].

3. Results

Table 1: Phytochemical Constituents of the Stem bark of *G. boveanum*

Secondary metabolite	ME	EE	n-HE
Alkaloids	+	+	-
Carbohydrate	+	+	-
Anthraquinones	-	-	-
Steroid	+	+	+
Triterpenes	+	+	-
Cardiac glycogen	+	+	-
Tannins	+	-	-
Flavonoid	+	+	-
Saponin glycoside	+	+	-

Keys: ME = Methanol Extract; EE = Ethyl acetate Extract; n-HE = n-Hexane Extract; + Presence, - Absent

Table 2: Sensitivity of the Microorganisms to varying Concentrations of the stem bark extracts of *G. boveanum*

Test Organism	Zone of Inhibition (mm)			
	ME (mg/ml) 50 25 12.5 6.25	**EE (mg/ml) 50 25 12.5 6.25**	**n-HE (mg/ml) 50 25 12.5 6.25**	**Ciprofloxacin 5µg/disc**
Staphylococus aureus	25 20 18 15	16 14 12 10	10 0 0 0	35
Bacillus subtilis	24 21 18 15	25 23 20 16	18 15 12 0	37
Candida albicans	0 0 0 0	0 0 0 0	0 0 0 0	0
Streptococcus agalactiae	0 0 0 0	0 0 0 0	0 0 0 0	32
Salmonella typhii	18 16 13 11	26 23 19 16	20 16 13 10	42
Shigella dysenteriae	18 15 11 0	23 20 17 12	16 10 0 0	40
Escherichia coli	20 18 16 14	18 14 12 0	13 10 0 0	35

Keys: ME = Methanol Extract; EE = Ethyl acetate Extract; n-HE = n-Hexane Extract

Table 3: MIC of the extracts of stem bark of *G. boveanum*

	MIC (mg/ml)		
Test Organism	**ME**	**EE**	**n-HE**
Staphylococus aureus	6.25	3.125	0
Bacillus subtilis	12.5	12.5	3.125
Salmonella typhii	6.25	3.125	3.125
Shigella dysenteriae	6.25	3.125	0
Escherichia coli	12.5	12.5	0

Keys: ME = Methanol Extract; EE = Ethyl acetate Extract; n-HE = n-Hexane Extract, MIC = minimum inhibitory concentration

Table 4: MBC of the extracts of stem bark of *G. boveanum*

	MBC (mg/ml)		
Test Organism	**ME**	**EE**	**n-HE**
Staphylococus aureus	12.5	6.25	0
Bacillus subtilis	25	25	6.25
Salmonella typhii	12.5	6.25	6.25
Shigella dysenteriae	12.5	6.25	0
Escherichia coli	25	25	0

Keys: ME = Methanol Extract; EE = Ethyl acetate Extract; n-HE = n-Hexane Extract, MIC = minimum inhibitory concentration

4. Discussion

The results of the phytochemical screening of the stem bark of *G. boveanum* are shown in Table 1. It indicates the presence of alkaloids, carbohydrate, steroids, flavonoids, tannins, saponin glycosides, cardiac glycosides and triterpenes. The n-hexane extract showed the presence of steroids only, while ethyl acetate and methanol extracts show the presence of alkaloid, carbohydrate, steroid, triterpenes, cardiac glycogen, saponin glycoside, and flavonoids. Anthraquinone is absent in all the three extracts, while tannins is absent in n-hexane and ethyl acetate extracts. The plant extracts demonstrated good number of vital secondary metabolites that are precursor for drug synthesis due to their activity, especially flavonoids which are good scavengers of radicals due to the reactivity of the hydroxyl group on them [13].

The antimicrobial activity of methanol, ethyl acetate and n-hexane extracts of the stem bark from *G. boveanum* was assayed against six species of bacteria including *Staphylococus aureus, Bacillus subtilis, Streptococcus agalactiae, Salmonella*

typhii, *Shigella dysenteriae*, *Escherichia coli* and (*Candida albicans*). The results of antimicrobial activity are presented in Table 2. The methanol and ethyl acetate extracts showed effective antibacterial activity against all bacteria except *S. Agalactiae* and fungi – *C. albicans*, while the n-hexane extract showed moderate antibacterial activity against all bacteria except *S. Agalactiae* and fungi – *C. albicans* which did not show activity. Generally, the antimicrobial properties of plant extracts are attributed to secondary metabolites such as alkaloids, tannins, saponins etc [14, 15]. These chemical constituents may be responsible for the observed antimicrobial effects of the extracts.

The results of the minimum inhibitory concentration (MIC) of methanol, ethyl acetate and n-hexane extracts are shown in Table 3. It revealed that ethyl acetate and methanol extracts could inhibit the growth of five out of the seven tested microorganisms while the n-hexane extracts could inhibit two out of the seven tested microorganisms.

The result of the minimum bactericidal concentration (MBC) of methanol, ethyl acetate and n-Hexane extracts are shown in Table 4. The MBC for n-Hexane extract is 6.25mg/ml for *B.subtilis* and *S.typhii*, the MBC for ethyl acetate extract shows that *S.aureus***,** *S.typhii* and *S.dysenteriae* was 6.25mg/ml and 25mg/ml for *B.subtilis* and *E.coli*. The MBC for Methanol extract shows that *S.aureus***,** *S.typhii* and *S.dysenteriae was 12.5mg/ml* and 25mg/ml for *B.subtilis* and *E.coli*, the highest MBC is 25mg/ml while the lowest was 6.25mg/ml.

5. Conclusion

Preliminary phytochemical screening revealed the presence of some secondary metabolites which might be responsible for the observed antimicrobial activity against the tested microorganisms. The results from this study justify the ethnomedicinal properties ascribed to the plant.

6. Acknowledgement

The authors are grateful to Mr. Ochigbo Celestine for providing the plant, Mallam Musa Mohammed for identifying and authenticating the plant. We are also thankful to Mallam A. M Shittu and Mr Mubarak Danbatta for their valuable technical assistance in antimicrobial screening and phytochemical analysis respectively.

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