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ANTIBACTERIAL ACTIVITY AND SCREENING FOR PHYTOCHEMICAL COMPONENTS FROM *Borreria Stachydea* [(DC) HUTCH. & DALZIEL]

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ABSTRACT
The aim of this present study was to determine the phytochemicals and antibacterial activity of Borreria stachydea whole plant using chloroform and methanol as the extracting solvents. The powdered sample was subjected into soxhlet extraction by hot continuous percolation. The extracts were concentrated at 40°C by vacuum evaporation and later exposed to air drying to give dried crude extracts. Phytochemical screening of the extracts was performed using standard procedures available in literature. Antibacterial activity of B. stachydea was determined using agar well diffusion method, while the Minimal Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC) of the extracts against bacteria were determined by broth dilution technique. Phytochemical screening of the extracts revealed the presence of tannins, flavonoids, carbohydrates, cardiac glycosides, terpenes and steroids. Both extracts were active against Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae and Proteus mirabilis. Comparatively, methanolic extract contained more phytochemicals but lower values of zones of inhibition than chloroform extract. At a concentration of 10 mg/ml, both methanolic extract and sparfloxacin (a standard antibiotic) used as control in this study exhibited similar antibacterial activity against Bacillus subtilis. The results of this investigation revealed that Borreria stachydea contains phytochemicals that are potential sources of antibacterial agents.
Keywords: Borreria stachydea, Extract, Phytochemical, Antibacterial, MIC, MBC.

INTRODUCTION

The discovery of plants as therapeutic agents started in ancient times and has continued to thrive even in modern days. Documentation of plants with medicinal values dates back as far as 78 AD (Alice, 1996). Research has proved that developing countries rely mainly on medicinal plants for treatment of their prevailing ailments especially in areas where hospitals are not readily accessible (Lambo, 1979). In Japan, China and Great Britain, the use of herbal remedies is prevalent due to side effects associated with the use of synthetic drugs and the rising cost of effective drugs (Chin *et al*., 2006; Rainer and Douglas, 2006).

Among the bioactive constituents in plants are alkaloids, tannins, flavonoids and phenolic compounds (Edeogal *et al*., 2005). If bioactive compounds are carefully isolated, purified and identified, they can act against diseases and infections of human and animals when properly utilized. Knowledge of the chemical constituents of any plant is desirable not only for discovery of therapeutic agents, but also because such information is useful for the discovery of new resources from such chemical substances (Sathish *et al*., 2013). Traditional uses of medicinal plants have witnessed gradual directional change leading to more complex and advanced modern drugs. Several modifications, improvement, sophistication and newer discoveries have continuously contributed to the type, quality, presentation and concept of medicinal preparation (Sathish *et al*., 2013). The emergence of drug resistant pathogenic bacteria and the need to mitigate side effects often associated with the use of synthetic antibiotics have led to increased quest for discovery of new effective molecules from plant sources (Fred, 2006).

Borreria stachydea (DC) commonly known in English language as "ants wheat" and in Hausa language as "alkamar tururuwa" is an erect, hairy and weedy herb, about 30-60 *cm* high with mauve flowers. It belongs to Rubiaceae family. This plant is used in traditional medicine to treat venereal diseases, inflammations, and gonorrhea in Birinin Magaji Local Government Area of Zamfara State. *Borreria* species possesses a wide variety of medicinal properties; however a few species have been screened for confirmation of their biological activities. Onoja and Ndukwe (2013) reported isolation of oleanolic acid from chloroform extract of *Borreria stachydea.*

The aim of the present study was to investigate the phytochemicals and antibacterial activity of *Borreria stachydea* whole plant Birinin Magaji village. To the best of our knowledge, this happens to be the first phytochemical screening and antimicrobial activity study of *B. stachydea* since no study has been carried out to determine the antibacterial potential of the plant obtained from this area before now.

MATERIALS AND METHODS

Collection of plant material

The whole plant of *B. stachydea* was collected fresh from Birnin Magaji village, in Zamfara State, Nigeria. Taxonomical identification was done at the Herbarium Unit of Biological Science Department, ABU, Zaria, Nigeria and its voucher specimen (number 2756) was deposited in the same Department.

The plant material was air dried under shade, segregated and pulverized by manually into a coarse powder by pounding them using wooden mortar and pestle. The pulverized plant material was stored in polythene bag and kept away from moisture until it was used for further analysis.

Preparation of extracts

The powdered sample of *B. stachydea* (791.94 *g*) was carefully packed into a soxhlet extractor and successfully extracted with chloroform and methanol by hot continuous percolation. The extracts were concentrated at 40 $^{\circ}C$ in vacuum using a rotary evaporator and later subjected to air drying to give dried crude extracts (Momoh *et al.*., 2015).

Phytochemical screening

The chloroform and methanol crude extracts were analyzed for the presence of carbohydrates, alkaloids, saponins, tannins, steroids, flavonoids, anthraquinones, cardiac glycosides and terpenes based on the standard procedures available in literature (Brain and Turner, 1975).

Test for carbohydrates

Molisch's test was used. Each extract (1 *g*) was dissolved in 5 *ml* of distilled water and heated in a water bath. The solution was filtered and 4 drops of Molisch's reagent was added to the filtrate. Conc. H_2SO_4 (3 *ml*) was added to the mixture from the side of the test tube to form a lower layer. A purple colour appearing at the interface indicates the presence of reducing sugar in the extract (Trease and Evans, 2002).

Test for alkaloids

Each extract (1 *g*) was dissolved in 2*N* hydrochloric acids. The mixture was filtered and the filtrate was divided into three equal portions. One portion was treated with three drops of Mayer's reagent, another portion was treated with equal amount of Dragondroff's reagent and the third portion was treated with equal amount of Wagner's reagent respectively. The lack of creamy precipitate, orange precipitate and brown precipitate indicated the absence of alkaloids in an extract (Salehi-Surmbghi *et al.*, 1992).

Test for saponins

Frosthing test was used to detect the presence or absence of saponins in the extracts. Extract (0.5 *g*) was dissolved in 10 *ml* of distilled water in a test tube and vigorously shaken for 30 *s* and allowed to stand. Lack of a honey comb like structure in the extracts was taken as a preliminary evidence for the absence of the saponins (Madziga *et al.*, 2010).

Test for tannins

Ferric chloride test was used to detect the presence or absence of tannins in the extracts. Extract (0.5 *g*) was dissolved in 10 *ml* of distilled water in a test tube and filtered. Three drops of 0.1% ferric chloride was added to the filtrate and observed for brownish green or blue-black precipitate. A blue-black precipitate or blue-black precipitate appearance indicates the presence of tannins in the extract (Segelman *et al.*, 1969).

Test for steroids and triterpenes

Libermann-Bruchard's test was used to detect the presence of steroids and triterpenes. Acetic anhydride (5 *ml*) was added to 5 *ml* of each extract in a test tube. Conc. H_2SO_4 (1ml) was added carefully down the side of the test tube. A pink colour appeared in the chloroform and methanolic extracts which later changed into a blue-green colour indicating the presence of steroids and triterpenes. (Umesh *et al.*, 2010)

Test for flavonoids

To 1 ml of each extract, 1ml of 10% lead acetate solution was added. The formation of a yellow precipitate was taken as a positive test for presence of flavonoids. Only the methanolic extracts gave a yellow precipitate indicating the presence of flavonoids in the extracts (Rohit, 2015).

Test for anthraquinones

Borntrager's test was used to detect the presence of anthraquinones. Extract (0.5 *g*) was taken in a dry test tube and 5 *ml* of chloroform was added and shaken for 5 min. The extract was filtered and the filtrate shaken with equal volume of 10% of ammonia solution. A pink violet or red colour in the ammoniacal layer indicates the presence of anthraquinones. No colour was observed in all the extracts suggesting the absence of anthraquinones. (Siddiqui and Ali, 1997).

Test for cardiac glycosides

Keller-Kiliani's test was used to detect the presence of cardiac glycosides. Each extract (0.5 *g*) was dissolved in 5 *ml* glacial acetic acid containing 1 drop of 5% ferric chloride solution in a test tube. The test tube was held at an angle of 45°and 1 *ml* of concentrated sulphuric acid was added carefully. All the two extracts showed purple ring at the interface indicating the presence of cardiac glycosides (Inalegwu and Sodipo, 2013).

Test microorganisms

Pure clinical bacteria isolates of *Staphylococcus aureus, Corynebacterium ulcerans, Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis* and *Pseudomonas aeruginosa* were obtained from Ahmadu Bello University Teaching Hospital, Zaria, Kaduna State, Nigeria. The bacteria were identified using the procedure described by Cheesbrough, (2002) and maintained in nutrient broth at a temperature of 6°*C*. The standardization was done by suspending the cultured bacteria into sterile screw capped bottles containing the nutrient broth. Normal saline was then gradually added and the dilution of test organisms continued until the turbidity marched that of Mac-Farland's scale by comparison, which corresponds to bacterial concentration of 1.5 x 10^8 CFU/mL (NCCLS, 1990).

Test for antibacterial activity

The antibacterial activity of *B. stachydea* was performed against the seven bacteria by agar well diffusion method (El-Mohmood, 2009). Each extract was dissolved in 10 *ml* of dimethylsulphoxide (DMSO) to obtain a concentration of 10 *mg/ml*. The sterilized medium (nutrient agar) (20 *ml*) was poured into a sterile petri dish, covered and allowed to cool and solidify. The medium was inoculated with 0.1 *ml* of the standardized bacteria culture (1.5 x 10^8 CFU/mL) and allowed to dry at 39 $^{\circ}C$ for 30 minutes. A standard cork borer of 6mm diameter was used to make a well at the centre of each inoculated plate and filled with 0.1 *ml* of 10 *mg/ml* of extract. The same procedure was used to prepare a sparfloxacin standard (positive control).

The plates were incubated at 37 °*C* for 24 *h* after which the zones of inhibition were inspected and measured using a transparent meter rule. The tests were carried out in triplicates and mean values were recorded.

Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentrations of extracts on bacteria were determined using broth dilution technique described by Krivoshan (1989). A solution of 10 *mg/ml* of each extract was serially diluted in two fold to obtain varying concentrations of 10 *mg/ml*, 5 *mg/ml*, 2.5 *mg/ml*, 1.25 *mg/ml* and 0.625 *mg/ml* using nutrient broth and later inoculated with 0.1 *ml* suspension of test organism. The inoculated tubes were incubated at 37 °*C* for 24 hours and thereafter observed for the presence of turbidity. The lowest concentrations of extract, which inhibited visible growth (showed no turbidity) were noted and considered as the minimum inhibitory concentrations.

Minimum Bactericidal Concentration (MBC)

Minimum Bacterial Concentration test was determined using the broth dilution contents from MIC test (Krivoshan, 1989). The contents were subcultured onto the media (plates) free of antibacterial agents and incubated at 37 °*C* for 24 hours. The lowest concentrations without colony were recorded as MBC.

RESULTS AND DISCUSSION

The results of phytochemical screening of chloroform and methanolic extracts of *Borreria stachydea* are presented in Table 1. The phytochemical constituents of chloroform extract are carbohydrates, cardiac glycosides, triterpenes and steroids while methanolic extract contained flavonoids and tannins in addition to carbohydrates, cardiac glycosides, triterpenes and steroids. Saponins, alkaloids and anthraquinones were absent in both extracts. From the results obtained, chloroform extracted four while methanol extracted six phytocompounds. This may be due to high solubility of most of these compounds in polar protic solvents such as methanol, than in nonpolar solvents such as chloroform, further emphasizing the fact the number of phytochemical

Table 1: Phytochemical constituents of chloroform and methanolic extracts of *Borreria stachydea*

S/No.	Phytochemicals	Chloroform Extract	Methanolic Extract
1	Tannins	–	+
2	Saponins	–	–
3	Steroids	+	+
4	Alkaloids	–	–
5	Triterpenes	+	+
6	Flavonoids	–	+
7	Carbohydrates	+	+
8	Anthraquinones	–	–
9	Cardiac glycosides	+	+

Key: + = Present, – = Absent

compounds extracted from plants are solvent dependent. The presence of some of these bioactive secondary metabolites has been associated with antibacterial properties (Reuben *et al*., 2008) and all the phytocompounds detected were known to exhibit medicinal and physiological activity (Sofowora, 1993).

Tannins have been used for traditional protection of inflamed surfaces of the mouth and treatment of catarrh, wounds, hemorrhoids and diarrhea (Ogunleye and Ibioye, 2013). Tannins have also been reported to possess antimicrobial properties and are therefore very useful in drug production. Steroids are hormones having biological activity to control a number of metabolic processes in the body. Natural and synthetic steroids are used in oral contraceptives and in the treatment of arthritis, Addison's disease, and certain skin ailments. Terpenes help to counter stress and anxiety, improve mood, promote alertness and memory retention. For example, limonene, a major terpene has been shown to destroy breast-cancer cells in lab experiments, and has powerful antimicrobial action that can kill pathogenic bacteria (Marth, 2013). Flavonoids are polyphenolic group of metabolites which exert antioxidant activity. Apart from antioxidant property, other biological functions of flavonoids include protection against allergies, inflammation, platelet aggregation, microbes, ulcers, hepatoxins virus and tumors (Barakat *et al*., 1993; Okwu and Omodamiro, 2005). More than 200 naturally occurring cardiac glycosides have been identified and the purified extracts or synthetic analogues have been used treat congestive heart failure and cardiac arrhythmia.

Table 2: Antibacterial activity of chloroform and methanolic extracts of *Borreria stachydea* at10 mg/ml

Test bacteria	Zone of inhibition[a] (mm)		
	Chloroform Extract	Methanolic Extract	Sparfloxacin
S. aureus	24	21	42
C. ulcerans	0	0	29
B. subtilis	29	27	29
E. coli	26	22	32
K. pneumoniae	24	20	40
P. mirabilis	24	21	27
P. aeruginosa	0	0	0

[a]Values are means of triplicate determinations

The result for antibacterial potential of *Borreria stachydea* extracts are presented in Table 2. Both extracts were able to inhibit *S. aureus, B. subtilis, E. coli, K. pneumoniae* and *P. mirabilis*. On the other hand, the two extracts did not inhibit *C. ulcerans* and *P. aeruginosa*. Methanolic extract however, showed the highest activity having inhibition zones ranging from 24 - 29 *mm.* The zones of inhibition of the chloroform extract and the positive control range from 20 - 27 *mm* and 27-42 *mm* respectively. According to Johnson and Case (Johnson and Case, 1995), zone of inhibition equal to or greater than 16 mm is associated with microbial susceptibility. In view of the above, the two extract have shown significant antibacterial activity against five bacteria.

Table 3: Minimum Inhibitory Concentration (MIC) of chloroform extracts of *Borreria stachydea* against test bacteria

Test bacteria	**Concentration**				
	10mg/ml	5mg/ml	2.5mg/ml	1.25mg/ml	0.625mg/ml
S. aureus	–	–	o•	+	++
C. ulcerans					
B. subtilis	–	–	o•	+	++
E. coli	–	–	o•	+	++
K. pneumoniae	–	–	o•	+	++
P. mirabilis	–	–	o•	+	++
P. aeruginosa					

Key: – = No inhibition, **o•** = Minimum inhibition, **+** = Moderate inhibition, **++** = Heavy inhibition

Interestingly, it was observed that the chloroform extract and the standard antibiotic used as control produced the same value of inhibition zone (29 *mm*) against *B. subtilis.* This means that sparfloxacin and chloroform extract of *Borreria stachydea* exerted similar pharmacological activity against *B. subtilis.* The results also revealed that *E. coli*, a well known multi-resistant bacterium was susceptible to both extracts while *P. aeruginosa*, another multi-resistant bacterium was resistant to both extracts and the control, further confirming its multi-resistance to plant extracts and drugs.

Table 4: Minimum Inhibitory Concentration (MIC) of methanolic extracts of *Borreria stachydea* test bacteria

Test bacteria	**Concentration**				
	10mg/ml	5mg/ml	2.5mg/ml	1.25mg/ml	0.625mg/ml
S. aureus	–	–	o•	+	++
C. ulcerans					
B. subtilis	–	–	o•	+	++
E. coli	–	–	o•	+	++
K. pneumoniae	–	–	o•	+	++
P. mirabilis	–	–	o•	+	++
P. aeruginosa					

Key: – = No inhibition, **o•** = Minimum inhibition, **+** = Moderate inhibition, **++** = Heavy inhibition

Results obtained from the determination of minimum inhibitory concentration are presented in Tables 3 and 4. Efficacy of antimicrobial agent is indicated by minimum inhibitory concentrations (MIC) (Tuntiwachwuttikul *et al.*, 2008) or 50% inhibitory concentrations (IC_{50}) value (Macias-Rubalcava *et al.*, 2008). The results indicated variability in the inhibitory concentrations of each extract for the various bacteria. Both extract showed activities at the concentrations of 0.625 *mg/ml*, 1.25 *mg/ml* and 2.5 *mg/ml* and were minimally inhibited at 2.5 *mg/ml*.

Table 5: Minimum Bactericidal Concentration (MBC) of chloroform extract of *Borreria stachydea* against test bacteria

Test bacteria	**Concentration**				
	10mg/ml	5mg/ml	2.5mg/ml	1.25mg/ml	0.625mg/ml
S. aureus	o•	+	+	+	+
C. ulcerans					
B. subtilis	–	o•	+	+	+
E. coli	–	o•	+	+	+
K. pneumoniae	o•	+	+	+	+
P. mirabilis	o•	+	+	+	+
P. aeruginosa					

Key: – = No colony growth, **o•** = Minimum colony growth, **+** = Colony growth

Table 6: Minimum Bactericidal Concentration (MBC) of methanolic extract of *Borreria stachydea* against test bacteria

Test bacteria	Concentration				
	10mg/ml	5mg/ml	2.5mg/ml	1.25mg/ml	0.625mg/ml
S. aureus	o•	+	+	+	+
C. ulcerans					
B. subtilis	–	o•	+	+	+
E. coli	o•	+	+	+	+
K. pneumoniae	o•	+	+	+	+
P. mirabilis	o•	+	+	+	+
P. aeruginosa					

Key: – = No colony growth, **o•** = Minimum colony growth, **+** = Colony growth

It was observed that inhibition of the extracts against test organisms increases with decrease in extract concentrations. Equally, MIC for the extracts (2.5 *mg/ml*) was higher than that of the control antibiotic, sparfloxacin (2 *mg/ml*). Table 5 showed the MBC of the chloroform extract of *Borreria stachydea*. The MBC for *B. subtilis* and *E. coli* was 5 mg/ml, while MBC for *S. aureus*, *K. pneumoniae* and *P. mirabilis* was 10 *mg/ml*. Table 6 displayed the MBC for methanolic extract of *Borreria stachydea.* The MBC for *B. subtilis* was 5 mg/ml, while *E. coli, S. aureus*, *K. pneumonia* and *P. mirabilis* had MBC of 10 *mg/ml*.

CONCLUSION

From the results of this study, it can be inferred that chloroform and methanol extracts of *Borreria stachydea* exhibited significant antibacterial activity against the test bacteria which could be as a result of the phytochemicals present in the plant. This research has further established the use of this plant for the treatment of bacterial disease (gonorrhea) by people of Birnin Magagi. Further study is required to isolate and explore individual bioactive compound that is responsible for the antibacterial activity.

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