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Antimicrobial Activity and phytochemical Screening of *Borreria verticillata* flower Bud Ethanolic Extract and Fractions

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Abstract

Historically Borreria verticillata has been used for the treatment of diarrhea/dysentery, headache, malaria and aids wound healing.Phytochemicalscreening of B. verticillata flower bud was conducted so as to determine the secondary metabolite. The plant material was percolated with 96% ethanol by serial exhaustive technique and fractionation was carried out with different solvents in order of their polarity, starting with n-Hexane, Chloroform and 60% Methanol. The fractions were subjected to phytochemical screening to determine the classes of secondary metabolites. The qualitative evaluation of the antimicrobial activity of the extract/fractions was accomplished using the agar well diffusion method for bacteria. The result showed that glycoside, terpenoids, steroids, alkaloids and flavonoids are present in all the fractions while phenolic compounds and tannins are absent in the chloroform fraction and phenolic compound, reducing sugars and tannins are absent in the n-hexane fraction. The result of the antimicrobial activity obtained from the flower bud of B. verticillata revealed that all the crude extract/fractions inhibited or exhibited antibacterial activity against Salmonella typhi, Staphylococcus aureus, Escherichia coli, Klebsiella sp. and Enterobacter sp. All the extract/fractions demonstrated antimicrobial activity against the test bacteria with 60% methanol fraction demonstrating the highest activity. *The results of this study validate the traditional antimoicrobial usage of B verticillata.*

Keywords: *Borreria verticillata, Ethanolic extract/fractions, antibacterial activity.*

INTRODUCTION

The international awareness of medicinal plants has increased in recent years, the World Health Organization (WHO) estimated that 80% of the population in developing countries rely on

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traditional medicine mostly plant based for primary health care, this is a behavior that predates civilization and it is found in every society irrespective of its level of development and sophistication (Yagoub, 2008). Most people in the rural areas of the world depend largely on herbs for treatment of several ailments because they constitute indispensable components for medical practice and due to its low cost, easy access, ancestral experience, safety, efficiency and less side effects (Yadav and Munin, 2011 and Ushie *et al*., 2013). Many commercially proven drugs used in modern medicine were initially used in crude form in traditional healing practices, it is estimated that plant materials have provided the models for 50% western drugs (Adamu *et al*., 2013). Plants used in traditional African system of medicine have been found to be active against a wide variety of micro-organisms and have always provided a good source for anti-infective agents and for therapeutic purpose to cure several disorders (Abubakar *et al*., 2017 and Ushie *et al*., 2013).

Recipes of plants are made from combination of different parts of two or more plants or from a single plant part, the parts used include roots, stem, stem bark, flower bud, flower, fruit, twigs, exudates and modified plant organs (Mahesh and Satish, 2008), they are ingested as decoctions, teas, infusion, pounded, soaked, squeezed and boiled, others are applied as ointments (Newman, 2008).

Borreria verticillata(*B. verticillata*) or *Spermacoce verticillata,* also known as broom vassourinha plant is a perennial shrubby false-button weed herb which has immense potential as a medicinal herb (Ushie *et al*., 2013). It is widely distributed in tropical areas in Africa, Asia and America. In Nigeria, Studies have confirmed that extracts from different parts of *B. verticillata* as well as its isolated compounds possesses diverse biological activities including antibacterial, analgesic, anti-inflammatory, antitumor, antidiarrheal, larvicidal, antioxidant, gastrointestinal, antimalarial, anti-ulcer, and hepatoprotectiveproperties and it has been commonly used effectively to cure skin diseases, toothache, diarrhea, headache, dyspepsia, leprosy, furuncles, ulcers, gonorrheal sores, bilharziasis and paralysis, with alkaloids and flavonoids as the major active compounds (Shajiselvin, 2010; Conserva and Ferreira, 2012; Ushie *et al*., 2013 and Abdullahi-Gero*et al.,* 2014). Therefore *B. verticillata* flower bud (aerial parts) is used as a traditional herb for its medicinal value (Ushie *et al*., 2013).

MATERIALS AND METHODS

Collection and Identification of Plant Materials

The flower bud was collected in October 2019from Botanical garden of the Bayero University Kano, Nigeria and identified at Herbarium section of the Department of Plant Biology, Bayero University Kano.The flower bud was air dried under the shade and powdered using pestle and mortar and transferred into airtight containers. Two hundred grams (200g) of powdered sample was percolated with 96% ethanol by serial exhaustive technique (Fatope *et al*., 1993). The extract was filtered into beakers using a Whatman No. 1 filter paper and evaporated to dryness, weighed and labeled.The crude ethanolic extract was fractionated with different solvents in increasing order of polarity starting with absolute n-Hexane, Chloroform and 60% Methanol as described by Oluwaseun *et al*. (2013).

Phytochemical screening of Extract/fractions

The extract/fractions were screened for the presence of bioactive agents according to the methods of Arunkumar and Muthuselvam (2009) and Ushie and Adamu, (2010).

Isolation of Test Organisms

The study population included children of 6 months to five years who reported to the hospital with diarrhea. A sterile container was used for the collection of stool samples while rectal swabs where collected when the stool is not available from the children. This was carried out by theresearcher with the assistance of a trained lab technician. The media used for the purpose of this study included MacConkey agar, Deoxycholate agar (DCA), Blood agar, and Salmonella-Shigella agar (SSA) and were prepared in accordance with manufacturer's instructions.All the samples were cultured and incubated at 37^{o} for 24 hours (Cheesebrough, 2010).

Biochemical Test

All plates that showed evidence of growth were subjected to standard bacteriologic biochemical tests which included: Catalase, Coagulase, Indole tests, Methyl red test, Vogas-prosekuer test, Citrate utilization test (IMViC), Oxidase test, Urease test, sugar fermentation, gas production and motility tests as described by Cheesbrough,, (2010) then advanced biochemical identification was carried out using Microgen identification kit (Microbact 24E) from Oxoid Laboratory Products and software following the manufacturer's instructions.

Susceptibility test

The agar well diffusion technique was used as described by Prescott,(2002),five (5) wells of 6mm diameter each were made on inoculated Muller Hinton agar using a sterile cork borer. The wells were filled with different concentrations of the extract/fractions; 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml and DMSO (as negative control) and are allowed to diffuse for about 2 hours. Discs of Gentamicin were used as positive control. The test plates were incubated at 37oC for 24hr. and the observed zones of inhibition measured.

Results

Table 1: Physical appearance and yield of extract/fractions from the flower buds of *Borreria verticillata* plant

Solvents	Extract colour	Extract/fractions Texture	Weight of plant material used (g)	Extract/fractions yield (g)	Percentages recovery(%)
Ethanol	brown	solid powder	200	5.037	2.515
n- hexane	Green	Soft powder	3.00	0.781	26.03
Chloroform	Green	Soft powder	3.00	0.654	21.80
60% methanol	Brown	Soft powder	3.00	0.600	20.00

Table 2: Preliminary phytochemicals of extract/fractions of *Borerriaverticillata* flower bud

Phytochemical compounds	Saponins	PC	Glycosides	Terpenoids	Steroids	Alk	Flavonoids	RS	Tan
Ethanol	+	+	+	+	+	+	+	+	+
Chloroform	+	-	+	+	+	+	+	+	-
n-hexane	+	-	+	+	+	+	+	-	-
60% Methanol	+	+	+	+	+	+	+	+	+

Key: PC- Phenolic compounds, Alk- Alkaloids, RS- Reducing sugar, Tan- Tannins, + Present – Absent

Table 3: Antibacterial activity of the extract/fractions of the flower bud of *B. verticillata*

Plant extract/fractions		Isolates	Concentrations (mg/ml)/ zone of inhibition (mm)				Controls	
			100	50	25	12.5	Gen	Dmso
96% ethanol		*S. aureus*	5.5±0.7	4±0.7	2±1.41	0±0.0	32	0
		E. coli	5±1.41	3.5±0.5	2±0.0	0±0.0	25	0
		Salmonella sp.	4±0.6	2±0.0	0±0.0	0±0.0	22	0
		Klebsiella sp.	5.5±0.5	3.5±0.5	1.5±0.5	0±0.0	22	0
		Enterobacter sp.	8.5±0.5	5±10	3.5±0.5	1.5±0.5	20	0
n-hexane		*S. aureus*	9.5±0.7	5.5±0.5	4±0.0	2±0.0	32	0
		E. coli	0±0.0	0±0.0	0±0.0	0±0.0	32	0
		Salmonella sp.	3.5±0.5	1.5±0.5	0±0.0	0±0.0	22	0
		Klebsiella sp.	5.5±0.5	2±0.0	1.5±0.5	0±0.0	22	0
		Enterobacter sp.	5±1.0	4±0.0	1.5±0.5	0±0.0	20	0
Chloroform		*S. aureus*	7.5±0.5	6.5±0.5	5.5±0.5	4.5±0.5	30	0
		E. coli	0±0.0	0±0.0	0±0.0	0±0.0	38	0
		Salmonella sp.	3±1.4	1.5±0.5	0±0.0	0±0.0	22	0
		Klebsiella sp.	4±0.0	2±0.0	1.5±0.5	1±0.0	20	0
		Enterobacter sp.	3.5±0.5	2.5±0.5	1.5±0.5	1.5±0.5	23	0
60% Methanol		*S. aureus*	6.5±0.5	5±1.0	3.5±0.5	1±0.0	34	0
		E. coli	0±0.0	0±0.0	0±0.0	0±0.0	36	0
		Salmonella sp.	4±0.0	1.5±0.5	2±0.0	0±0.0	24	0
		Klebsiella sp.	5±1.0	3.5±0.5	2±0.0	0±0.0	22	0
		Enterobacter sp.	5.5±0.5	4±0.0	1.5±0.5	0±0.0	20	0

Key:*S. aureus- Staphylococcus aureus, E. coli- Escherichia coli,* ±- Mean standard deviation

DISCUSSION

Extraction of secondary metabolites from the flower bud of *B. verticillata* using 96% ethanol, hexane, chloroform and 60% methanol yielded 5.037g (2.515%), 0.781g (26.03%), 0.654g (21.80%) and 0.60g (20.00%). (see Table 1).

Table 2 shows the result of phytochemical screening, it is depicted that Saponins, glycoside, terpenoids, alkaloids and flavonoids are present in all the fractions while phenolic compounds and tannins are absent in the chloroform fraction and phenolic compound, reducing sugars and tannins are absent in the n-hexane fraction, all the phytochemical compounds are present in the crude ethanol and 60% methanol fractions.These phytochemicals have been reported by Mohammed *et al., (*2016) for antimicrobial activity with Alkaloids, tannins, flavonoids, and phenols as the main phytochemical constituents. Ushie and Adamu(2010), also reported the same phytochemicals in the leaf extract/fractions of *B.verticillata* in which hexane, ethyl acetate, acetone, chloroform and methanol were used in the extraction. This indicates that both the flowers and leaves are similarin their phytochemical content. This similarity is not surprising since flowers are modified leaves (Anele *et al., 2014)*.Steroid was detected in all the fractions, this is contrary with the work of Okwu in 2004, were it was found only in ethyl acetate fraction of the flower bud.

The results of the antimicrobial activity (see Table 3) revealed that all the extract/fractions showed antibacterial activity against *Salmonella typhi*, *Staphylococcus aureus*, k*lebsiella* sp., *Enterobacter* sp. except for *Escherichia coli* that was susceptible to only the extract and resistant to the fractions.This is in line with the findings of Balde (2015), that the flower buds were active against different types of bacteria and fungi, and was more active than the whole plant and the root. The antibacterial effect and spectrum of the extract/fractions of the flower buds showed similar activity therefore, this indicates that the plant has antimicrobial activity notwithstanding the solvent used for the extraction.

CONCLUSION

From the result of this study, it can be concluded that *B. verticillata*presents potential of producing new drugs for the treatment of diarrhoea in children. Further research should be carried out on this plant to determine its safety for consumption

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