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Phytochemical Screening and Antibacterial Activity of *Brillantaisia patula* Leaf

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ABSTRACT

Brillantaisia patula is a medicinal plant used for different ailments in Africa. Phytochemical constituents and antibacterial potential of the plant were investigated. *In vitro* antibacterial activity using agar-well diffusion method was carried out against *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 24212), *Proteus hauseri* (ATCC 13315), *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* (ATCC 38218). The phytochemistry of both methanol and ethanol extracts of *Brillantaisia patula* leaf revealed the presence of alkaloids, glycosides, terpenoids, steroids, flavonoids, tannins, steroids, flavonoids and saponins. Terpenoids and flavonoids were found in the methanolic extract but not in the ethanolic extract while steroid was found in the ethanolic extract but not in the methanolic extract. The ethanolic extract was active against all the five pathogenic bacteria while the methanolic extract inhibited all the test bacteria but *Staphylococcus aureus*. Ethanolic extract zones of inhibition ranged from 15.3±0.6 to 20.7±0.6 mm, whereas methanolic extract zones of inhibition ranged from 18.0±1.0 to 30.0±1.0 mm. The Minimum Inhibitory Concentration (MIC) of ethanolic extract ranged from 25 to 200 mg mL⁻¹ while that of methanolic extract was from 50 to 100 mg mL⁻¹. The Minimum Bactericidal Concentration (MBC) of methanolic extract ranged from 100 to 200 mg mL⁻¹. The least MBC value (50 mg mL⁻¹) of ethanolic extract was against *Escherichia coli* while the highest value (>200 mg mL⁻¹) was against *Proteus hauseri*. Leaf of *Brillantaisia patula* could be a novel source of antibacterial agent (s) that might have broad spectrum activity.

Key words: Medicinal plant, agar-well diffusion, *in vitro*, pathogenic bacteria, minimum inhibitory concentration and minimum bactericidal concentration

INTRODUCTION

Importance of plants to man's existence cannot be over emphasized. Plants are used as sources of foods and medicines since the dawn of civilization (Sofowora, 1993). Many indigenous plants; trees, shrubs, herbs, twigs and leafy vegetable are taken as food, spices or used for medicinal purposes in Nigeria (Nwaogu *et al.*, 2007). In Africa, where the larger percentage of the populace lives in rural area, plants are used extensively in traditional health care delivery in combating

communicable and non-communicable diseases. According to World Health Organisation (WHO), over 80% of the world's population depends on medicinal plants for their health care needs (Upadhyay *et al.*, 2011). Plant-derived medicines are relatively cheaper than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment (Abubakar, 2009). Many of these plants are readily available in rural areas, thus making traditional system of medicine relatively cheaper than the orthodox medicine. The medicinal value of plants depends on their chemical constituents that provide a definite physiological action on the human body (Kumar *et al.*, 2009). These bioactive constituents are tannin, flavonoid, alkaloids, phenol compounds, saponin, glycoside, anthraquinones etc. (Adegoke *et al.*, 2010; Duke, 1995; Kamba and Hassan, 2010; Ujowundu *et al.*, 2010). The knowledge of chemical constituents of plant is necessary for the discovery of therapeutic agents of importance and their precursors. Many plant extracts are known to have antimicrobial properties. In recent years, several studies have evaluated antimicrobial activity of crude plant extracts (Adegoke *et al.*, 2010; Ayepola, 2009; Abubakar, 2009; Habtamu *et al.*, 2010; Chhetri *et al.*, 2008; Rani *et al.*, 2008; Kamba and Hassan, 2010; Vinothkumar *et al.*, 2010). *Brillantaisia patula* belongs to the family Acanthaceae; it is a shrubby plant of about 3 m height and can be found in Nigeria, Togo, west Cameroon and across Uganda and Angola. The leaves are used to take care of yaws and rheumatism, the decoction is taken to ease childbirth, menstrual pain and stomach ache. It has been reported to have antiplasmodial and analgesic potentials (Makambila-Koubemba *et al.*, 2011; Mbatchi *et al.*, 2006). The present study was carried out to determine the chemical and antimicrobial activity of leaf extracts of *Brillantaisia patula*.

MATERIALS AND METHODS

Plant material: The leaves of *Brillantaisia patula* were collected during the months of December (2010)-February (2011) at Oke-Ola, Ilaro, Yewa-South local government of Ogun State, Nigeria. The plant materials were authenticated by Mr. P.O. Bankole, a botanist in Biology unit of Science Laboratory Technology Department, Federal Polytechnic, Ilaro, Nigeria. The plant materials were cleaned with distilled water, shade-dried at room temperature ($27\pm 2^{\circ}\text{C}$) and pulverized using Kenwood electric blender (Kenwood Ltd., Harvart, United Kingdom). The resulting powder was kept in air-tight container ($27^{\circ}\text{C}\pm 2$) until required.

Preparation of plant extracts: Crude solvent extraction of *Brillantaisia patula* leaves was carried out using modified method of Omale *et al.* (2010). The powdered leaves were soaked in ethanol (90%) and methanol (90%) and filtered after 72 h. The filtrates were evaporated to dryness using rotary evaporator.

Bacterial strains: The following gram-positive and gram-negative species were the test organisms used: *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 24212), *Proteus hauseri* (ATCC 13315), *Pseudomonas aeruginosa* (ATCC 27853) and *Escherichia coli* (ATCC 38218) were used for *in vitro* antibacterial studies. All the stock cultures were obtained from the National Institute of Medical Research (NIMR) Yaba, Lagos State, Nigeria.

Phytochemical screening: Qualitative determination of phytochemical constituents of the plant extracts was carried out using various methods. Iyengar (1995) methods were followed for the determination of tannin, saponin and reducing sugar. Methods of Siddiqui and Ali (1997) were

adopted for alkaloids, glyceroids, steroid, terpenoids, flavonoid and anthroquinones. Phlobatanin was determined according to the method of Kumar *et al.* (2009).

Purity test: Purity test was carried out on the crude extracts before use. Pour plate method and plate count agar were used for the test. The plates were incubated at 37°C for 24 h. The absence of growth was used as indicator of sterility of the extracts.

Determination of antimicrobial activity: The study was performed under strict aseptic condition. The test organisms (bacteria) were sub-cultured into nutrient broth and incubated at 37°C for 24 h. Normal saline was added to adjust the turbidity to 0.5 McFarland standards which corresponds to 10^8 cells mL⁻¹ (Habtamu *et al.*, 2010; NCCLS, 1990). Modified agar well diffusion method of Habtamu *et al.* (2010) was adopted. The plates were in triplicate and were left on the working bench for 30 min to allow for diffusion before incubation at 37°C for 24 h (Esimone *et al.*, 1998). The diameter of the resulting zone of inhibition was measured using transparent millimeter calibrated ruler.

Minimum Inhibitory Concentration (MIC): The methods described by El-Mahmood *et al.* (2008) and Vinothkumar *et al.*, 2010 were used. The following concentrations; 200, 100, 50 and 25 mg mL⁻¹ were prepared using two-fold serial dilution. The tubes were incubated at 37°C for 24 h and observed for visible growth. The lowest concentration at which no detectable bacterial growth occurred was considered as Minimum Inhibitory Concentration (MIC).

Minimum Bactericidal Concentration (MBC): The minimum bactericidal concentration of the extract was determined by selecting tubes that showed no growth during MIC determination. A loopful was taken from the test tubes and inoculated on sterile Muller Hinton agar by streak plate method in triplicates. The plates were incubated at 37°C for 24 h. The lowest concentration of the extracts that showed no colony growth on the solid medium was regarded as minimum bactericidal concentration (Mann *et al.*, 2008).

Statistical analysis: The results of zones of inhibition were presented as mean of replicates and Standard Deviation (SD).

RESULTS

Alkaloids, glycosides, terpenoids, steroids, flavonoids, tannins and saponins were present in either or both extracts. Alkaloids, glycosides, tannins and saponins were present in both extracts. Terpenoids and flavonoid were present in the methanolic extract but not in ethanolic extract. Steroid was present in ethanolic extract, not in methanolic extract. Phlabotanins and reducing sugar were absent in both extracts (Table 1).

The susceptibility of the test organisms to the crude extracts on the basis of zones of inhibition varied according to organisms and extraction solvents. Methanolic and ethanolic extracts showed varying degrees of activity against the test organisms. *Brillantaisia patula* leaf ethanolic was active against the five test organisms; *Enterococcus faecalis*, *Proteus hauseri*, *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The methanolic extract showed activity against all the test organisms except *Staphylococcus aureus*. Standard antibiotic (pefloxacin) only showed activity against *Enterococcus faecalis*, *Staphylococcus aureus* and *Pseudomonas*

Table 1: Phytochemical screening of *Brillantaisia patula* leaf

Active constituents	Ethanollic extract	Methanollic extract
Alkaloids	+	+
Glycosides	+	+
Terpenoids	-	+
Steroids	+	-
Flavonoids	-	+
Tannins	+	+
Saponins	+	+
Phlabotanins	-	-
Reducing sugar	-	-

+: Present; -: Absent

Table 2: Antimicrobial activity of *Brillantaisia patula* leaf extracts

Microorganisms	Mean zones of inhibition (mm) and standard deviation			
	E. extract Mean±SD	M. extract Mean±SD	PFX (5 µg mL ⁻¹) Mean±SD	Control Mean±SD
<i>Enterococcus faecalis</i> ATCC 24212	29.7±0.6	22.3±0.6	30.3±1.5	0
<i>Proteus hauseri</i> ATCC 13315	15.3±0.6	18.0±1.0	R	0
<i>Escherichia coli</i> ATCC 38218	31.3±1.2	26.3±1.5	R	0
<i>Staphylococcus aureus</i> ATCC 25923	20.7±1.2	0	26.7±0.6	0
<i>Pseudomonas aeruginosa</i> ATCC 27853	23.3±0.6	30.0±1.0	10.0±1.0	0

E: Ethanollic; M: Methanollic; PFX: Pefloxacin; R: Resistant

Table 3: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of ethanollic extract of *Brillantaisia patula* leaf

Microorganisms	Ethanollic extract (mg mL ⁻¹)					MIC (mg mL ⁻¹)	MBC (mg mL ⁻¹)
	12.5	25.0	50.0	100.0	200.0		
<i>Enterococcus faecalis</i> ATCC 24212	+	+	-	-	-	50	100
<i>Proteus hauseri</i> ATCC 13315	+	+	+	+	-	200	>200
<i>Escherichia coli</i> ATCC 38218	+	-	-	-	-	25	50
<i>Staphylococcus aureus</i> ATCC 25923	+	+	-	-	-	50	100
<i>Pseudomonas aeruginosa</i> ATCC 27853	+	+	-	-	-	50	100

+: Growth; -: No growth

aeruginosa. The zones of inhibition of extracts ranged from 15.3±0.6 and 31.3±1.2 mm. Ethanollic extract showed the highest and least zones of inhibition against *Escherichia coli* and *Proteus hauseri*, respectively. As shown in Table 2, extraction solvents (control) were not active against the test organisms. The zones of inhibition of methanollic extract ranged from 18.0±1.0 and 30.0±1.0 mm.

Ethanollic extract inhibited *Enterococcus faecalis*, *Proteus hauseri*, *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* at minimum concentration of 50, 200, 25, 50 and 50 mg mL⁻¹, respectively. Ethanollic extracts demonstrated bactericidal activity against *Enterococcus faecalis* and *Pseudomonas aeruginosa* at minimum concentration of 100 mg mL⁻¹. Ethanollic extract also showed MBC against *Escherichia coli* and *Proteus hauseri* at concentrations of 50 and >200 mg mL⁻¹, respectively (Table 3).

Table 4: Minimum inhibitory concentration of (MIC) and minimum bactericidal concentration (MBC) of methanolic extract of *Brillantaisia patula* leaf

Microorganisms	Methanolic extract (mg mL ⁻¹)					MIC (mg mL ⁻¹)	MBC(mg mL ⁻¹)
	12.5	25.0	50.0	100.0	200.0		
<i>Enterococcus faecalis</i> ATCC 24212	+	+	+	-	-	100	100
<i>Proteus hauseri</i> ATCC 13315	+	+	+	-	-	100	200
<i>Escherichia coli</i> ATCC 38218	+	+	-	-	-	50	100
<i>Pseudomonas aeruginosa</i> ATCC 27853	+	+	+	-	-	100	100

+: Growth; -: No growth

Methanolic extract of *B. patula* inhibited *Enterococcus faecalis*, *Proteus hauseri* and *Pseudomonas aeruginosa* at a minimum concentration of 100 mg mL⁻¹ and *Escherichia coli* was inhibited at minimum concentration of 50 mg mL⁻¹ as shown in Table 4. Methanolic extract demonstrated bactericidal activity against *Enterococcus faecalis*, *Escherichia coli* and *Pseudomonas aeruginosa* at minimum concentration of 100 mg mL⁻¹ (Table 4). Methanolic extract also showed MBC at a concentration of 200 mg mL⁻¹ against *Proteus hauseri* (Table 4).

DISCUSSION

The study revealed the antibacterial potential of *Brillantaisia patula* and its phytochemical constituents. The presence of notable phytochemicals; alkaloids, glycosides, terpenoids, steroids, flavonoids, tannins and saponins in the plant leaf might have been responsible for its uses in traditional health care. These bioactive constituents (secondary metabolites) have been associated with antimicrobial activities in mammalian cells (Mishra *et al.*, 2009; Nweze *et al.*, 2004; Sofowora, 1993). Several phenolic compounds like tannins are potent inhibitors of many hydrolytic enzymes such as proteolytic macerating enzymes used by plants pathogens (Kamba and Hassan, 2010). Non-toxic glycoside can also be hydrolysed to release phenolic compounds that are toxic to microbial pathogens. These bioactive compounds exert antimicrobial activity through different mechanisms. Tannins are known to form irreversible complexes with proline-rich protein (Shimada, 2006), resulting in the inhibition of microbial cell protein synthesis. Plants with tannins as their main components are astringent in nature and are used for treating intestinal disorder such as diarrhoea and dysentery (Dharmananda, 2003). Tannins also have anticancer activity and can be used in cancer prevention (Okuda and Ito, 2011), thus *Brillantaisia patula* has the potential as a source of vital bioactive compounds for the management of cancer. Cardiac glycosides have good potentials in the field of cancer therapy and treatment of heart failure (Prassas and Diamandis, 2008; Schoner and Schein-Bobis, 2007) are naturally cardioactive drugs used in the treatment heart failure and cardiac arrhythmia (Agbafor and Nwachukwu, 2011). The extracting solvents played important role in the phytochemical constituents of the extracts as revealed by the variation in the phytochemical constituents of methanolic extract and ethanolic extract of the same plant. This variation could be due to difference in the extraction ability of the solvents. This finding was in agreement with the work of Egharevba *et al.* (2010).

The antibacterial assays showed that extracts of *Brillantaisia patula* leaf have antipathogenic bacteria potential. Both extracts showed significant antibacterial activity, but ethanolic extract was more active based on the number of test bacteria inhibited. The two extracts showed varying zones of inhibition. Habtamu *et al.* (2010) also reported difference in antibacterial activity with different extraction solvents. The antibacterial activity of the plant could be due to phytochemicals identified

in the extracts. Various works have reported antimicrobial activity of different medicinal plants (Adegoke *et al.*, 2010; Ayepola, 2009; Chhetri *et al.*, 2008; Abubakar, 2009; Habtamu *et al.*, 2010; Rani *et al.*, 2008; Kamba and Hassan, 2010). The crude extracts compared favourably with the standard antibiotic (pefloxacin) based on the zones of inhibition but crude extract were better in terms of number of test bacteria inhibited. Inability of methanolic extract to inhibit *Staphylococcus aureus* might be due the concentration of extract used for the antibacterial assay.

The MIC and MBC assays showed the highest MIC and MBC values against *Proteus hauseri*. The bacteria was less susceptible to the crude extracts, the organism might also be carrying gene that could partially hydrolyse part of the active constituents of the extracts. *Escherichia coli* was the most susceptible with least MIC and MBC values, *Brillantaisia patula* could be a potential source of antibacterial agents for the management of enteric disorder. The *in vitro* antibacterial activity of *Brillantaisia patula* might not be enough to justify its potential, there is need for the *in vivo* antibacterial activity to be evaluated.

CONCLUSION

The methanolic and ethanolic extracts of *Brillantaisia patula* contained a good number of bioactive chemical constituents including alkaloids, glycosides, terpenoids, steroids, flavonoids, tannins and saponins. Extraction solvents played important role in the phytochemical constituents of the extracts. Based on the number of phytochemicals identified in the extracts, methanol was a better solvent. Ethanolic extract was more active against all the test organisms. Leaf extract of *Brillantaisia patula* could be a novel source of antibacterial agent (s) that might have broad spectrum activity.

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