

## Full Length Research Paper

## Phytochemical and antimicrobial activity of the stem-bark of *Gardenia aqualla* Stapf & Hutch (Rubeaceae)

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*Gardenia aqualla* Stapf & Hutch (Rubeaceae) is a plant belonging to the family Rubeaceae. Preliminary phytochemistry carried out on the methanolic (ME) extract of the stem bark revealed the presence of steroid, carbohydrates, anthraquinones, saponins, triterpenes, tannins, cardiac glycoside and flavonoid while the petroleum ether (PE) extract of the stem bark revealed the presence of steroid, triterpenes and flavonoids. The PE extract and the ME were evaluated for antimicrobial screening using agar diffusion and broth dilution method on the following clinical isolates; *Staphylococcus aureus*, *Enterococcus aerogenes*, *Escherichia coli*, *Salmonella typhi*, *Shigella dysenteriae* and the fungi *Trichophyton rubrum*, *Candida albicans* and *Microsporum spp.* Both extracts were active against *S. aureus*, *E. aerogenes*, *E. coli*, *S. typhi* and *S. dysenteriae* with minimum inhibitory concentration (MIC) between 3.13 and 6.25 mg/ml and 1.25 to 2.25 mg/ml for PE and ME extracts, respectively and minimum bactericidal concentration (MBC) ranged between 12.50 to 25.00 mg/ml and 2.50 to 5.00 mg/ml for PE and ME extracts, respectively. Both extracts showed no antifungal activity. The antibacterial activity of both extracts may be due to the presence of the secondary metabolites present. This study thus justifies the use of this plant in traditional medicine.

**Key words:** *Gardenia aqualla*, Rubeaceae, phytochemistry, antimicrobial activity.

### INTRODUCTION

Diseases caused by bacteria, viruses, fungi and other parasites are major causes of death, disability, social and economic disruption for millions of people (UNAIDS/WHO,

2008). Despite the existence of safe and effective interventions, many people lack access to preventive and treatment care. With the development of new antimicrobial

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agents, microbes developed the ability to elude our best weapons and to counterattack with new survival strategies. Antibiotic resistance occurs at an alarming rate among all classes of mammalian pathogens. *Pneumococci* resistant to penicillin and *Enterococci* resistant to vancomycin have become common. Even *Staphylococcus aureus* strains resistant to vancomycin have appeared (Longo et al., 2012). Over 9.5 million people die each year due to infectious diseases, and majority of the death, if not all, occur in developing countries (WHO, 2008). With the current increasing trends of multidrug resistance among emerging and re-emerging bacterial pathogens to the available modern drugs or antibiotics (Abiramasundari et al., 2011), it is necessary that the search for newer antibiotic sources be a continued process. Plants are the cheapest and safer alternative sources of antimicrobials (Sharif and Banik, 2006; Doughari et al., 2007).

The plant *Gardenia aqualla* is a shrub that grows up to 3 m high; of the savanna; in Senegal to North and South Nigeria, and in Sudan and Ubangi (Burkill, 1985). In tree-form the branches tend to project horizontally. Medicinally, the leaf is used to treat leprosy (Watt and Breyer-Brandwijk, 1963), roots used to treat oral infections and fruits for ear infection (Burkill, 1985), infusion of the stem bark is used to treat bowel disorder in Northern Nigeria. This work was embarked upon to determine its antimicrobial activity as a way of contributing to the search for antimicrobials in plants.

## MATERIALS AND METHODS

### Plant collection and identification

The plant sample was collected in the outskirt of Ahmadu Bello University, Zaria (located on latitude 11° 15'N to 11° 3'N of the equator and longitude 7° 30'E to 7° 45'E of Greenwich Meridian) (Idowu and Yetunde, 2012), Kaduna State, Nigeria in August, 2008. The plant was authenticated in the Herbarium of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria, by comparison with a herbarium specimen (voucher specimen 1895).

### Preparation and extraction of plant material

The stem-barks were removed and air-dried under shade and powdered using mortar and pestle. Four hundred grams (400 g) was defatted exhaustively with petroleum ether (60 to 80°C) and later with 95% methanol in a soxhlet extractor. The extracts were concentrated in vacuume to solid residue (Brain and Turner, 1975). It was then stored in a tight clean container at room temperature before use.

### Phytochemical screening

Phytochemical screening was carried out on the petroleum ether and methanolic extract of the stem bark of *G. aqualla* using standard procedures of analysis (Harborne, 1998; Sofowora, 1993;

Trease and Evans, 2002).

### Test organisms

Clinical isolates viz; *Staphylococcus aureus*; *S. pyrogenes*; *Corynebacterium ulcerans*; *Escherichia coli*; *Salmonella typhi*; *Shigella dysenteriae*; *Enterococcus aerogenes*; *Pseudomonas aeruginosa*; *Klebsiella pneumonia* and the fungi *Trichophyton rubrum*, *Microsporum sp.* and *Candida albicans* were obtained from Ahmadu Bello University Teaching Hospital, Zaria-Kaduna, Nigeria. All the micro-organisms were checked for purity and maintained in slants of agar.

### Cultivation and standardization of test organism

A loop full of each of the test organisms was taken from the agar slant and sub cultured into test tubes containing 20 ml of sterile nutrient agar (for bacteria) and sabouraud dextrose agar medium (for the fungi). The test tubes were then incubated for 24 h at 37°C for bacteria and 27°C for 48 h for the fungi. The growth culture was standardized using sterile normal saline to obtain a density of 10<sup>6</sup> cfu/ml for bacteria. A sporulated test fungal spores was harvested with 0.05% Tween 80 in sterile normal saline and standardized to 10<sup>6</sup> spores/ml.

### Preparation of culture media

The prescribed quantities of the dehydrated bacteriological culture media was weighed and hydrated with distilled water according to the manufacturers specification. Where necessary, gentle heat was applied to aid dissolution and the resultant suspensions were dispensed into clean bottles and sterilized at 121°C for 15 min in an Adelphi bench autoclave.

### Assay for antibacterial activity

The antibacterial screening was carried out using the agar diffusion method (Lino and Deogracious, 2006). The test bacteria were first inoculated into tubes of nutrient broth separately and incubated at 37°C for 18 h. Each of the cultures was then adjusted to 0.5 McFarland turbidity standard and (0.2 ml) inoculated onto Mueller Hinton agar (MHA, Oxoid) in petri plates (diameter: 15 cm). A sterile cork borer was then used to make wells (6 mm diameter) for the extract on each of the plates containing cultures of the different test organisms. The extracts were separately re-dissolved in dimethyl sulphoxide (DMSO) to obtain initial concentrations of 50 mg/ml. 0.5 ml of each of the extract was then introduced into the wells using sterile Pasteur pipettes. 0.5 ml of DMSO only was introduced in another well to serve as negative control. Wells containing the standard antimicrobials sparflloxin and fluconazole (0.5 mg/ml) were included as positive control. The culture plates were allowed to stand on the working bench for 30 min for pre diffusion and were then incubated at 37°C for 24 h. After 24 h, antibacterial activity was determined by measurement of diameter zones of inhibition (mm) (against the test organisms) around each of the extracts and the antibiotics (Lino and Deogracious, 2006).

### Minimum inhibitory concentration (MIC)

This was done using broth dilution method (Ajaiyeoba et al., 2003).

**Table 1.** The phytochemical constituents present in the Petroleum ether and methanolic extract of *G. aqualla*.

Constituents	Inference	
	PE	ME
Carbohydrate	-	+
Anthraquinones	-	+
Saponin	-	+
Steroid	+	+
Triterpenes	+	+
Tannins	-	+
Alkaloids	-	-
Cardiac glycosides	-	+
Flavonoid	+	+

+ = present; - = absent

In this method, 10 ml nutrient broth (prepared according to manufacturer's specifications) was dispensed into test tubes and sterilized at 121°C for 10 min and allowed to cool. McFarland's turbidity standard scale number 0.5 was prepared to give a turbid solution. Normal saline was inoculated with each of the test micro-organism and incubated at 37°C for 6 h to make a turbid suspension of the micro-organisms. After incubations, dilution of the micro-organism in DMSO was done until the turbidity ( $1.5 \times 10^6$  cfu/ml) matched that of the McFarland scale by visual comparison. Two fold serial dilution of the extract in the broth was done to obtain the following concentrations 100, 50, 25, 12.5, 6.25, and 3.13 mg/ml. From the suspension of the micro-organism in DMSO, 0.1 ml was inoculated into the different concentrations of the extract in the nutrient broth. The broths were incubated at 37°C for 24 h (for the bacteria) and 27°C for 48 h (for the fungi) after which the test tubes were observed for turbidity. The lowest concentration of the extract in the broth which shows no turbidity represents the MIC. The results after 24 and 48 h were recorded.

#### Minimum bactericidal concentration (MBC)

Mueller Hinton agar was prepared according to manufacturer's instruction, sterilized at 121°C for 15 min. It was poured into sterile Petri-dishes. The plates were allowed to cool and solidify. The contents of the MIC test tubes in the serial dilution which did not show any growth were then sub-cultured onto the prepared plates and the plates were then incubated at 37°C for 24 h (for the bacteria) and 27°C for 2 to 7 days (for the fungi) after which the plates were observed for growth. The plate without growth represents the minimum bactericidal concentration. After 24 h, the results were recorded (Ajaiyeoba et al., 2003).

## RESULTS

The methanolic extract of the stem bark of *G. aqualla* contained more methanol soluble phytochemicals (30.14%) than the petroleum ether (10.2%). The PE extract contained only steroid, triterpenes and flavonoid while the methanolic extract contained anthraquinones,

saponins, flavonoid, tannins, steroid and cardiac glycoside (Table 1). The antibacterial activities showed that the plant had a wide spectrum of activity against the bacteria tested (Table 2). The PE extract showed activity against all the strains of bacteria with much activity against *E. coli* with (27 mm) but none against the fungi isolates. The ME showed highest zone of inhibition. Both extracts showed lower zone of inhibition against the test bacteria compared to that of sparfloxin. The MIC of the ME extract (between 1.25 to 2.25 mg/ml) was lower than that of PE extract (between 3.125 to 6.250 mg/ml) indicating that the ME extract has higher activity than the PE extract (Table 3). The MBC ranged between 2.5 to 5.0 mg/ml and 12.5 to 25 mg/ml for the ME and PE extracts, respectively.

## DISCUSSION

It has been reported that the antibacterial activity depends on the total saponins and tannins content present in the plant extract (Avto et al., 2006). It has also been proposed that bactericidal effect of phenolic compounds viz tannins and flavonoids in plants are known to form complexes with peptidoglycans, sterols and other cell wall components resulting in cell leakage (Aboaba et al., 2006). Several reports are available in support of antimicrobial activity of saponins against bacterial and fungal pathogens (Mandal et al., 2005; Gopish and Kannabiran, 2008). Thus presence of saponins, tannins and flavonoids in the methanolic extract could account for the antibacterial activity of this extract while that for the petroleum ether extract can be due to the presence of flavonoid.

In view of the fact that prevalence of *S. aureus* resistant strains to conventional antibiotics has increased to high levels of some hospitals (Usman et al., 2007), *Salmonella* frequently causes food-borne illnesses in addition to typhoid and paratyphoid infection (Arshad et al., 2010) and *E. coli* which is one of the cause of urinary tract infection and accounts for approximately 90% of first urinary tract infection in young women (Usman et al., 2007), the methanolic extract could serve as a remedy to such resistance and a cure to food-borne illnesses and urinary tract infections since the extract has also showed the same level of activity.

## Conclusion

*Gardenia aqualla* is known to be medicinally important in several aspects. This study revealed that the stem bark of this plant has several active phytochemicals that are inhibitory to several microorganisms. The significant activity of the methanolic extract against microorganisms

**Table 2.** The zone of inhibition (mm) of methanolic and petroleum ether extract of stem-bark of *G. aqualla* together with sparfloxin and fluconazole on test organisms.

Micro-organism	Zone of Inhibition (mm)			
	PE	ME	Sparfloxin	Fluconazole
<i>Staphylococcus aureus</i>	20	30	44	-
<i>Enterococcus aerogenes</i>	25	37	38	-
<i>Escherichia coli</i>	27	29	30	-
<i>Salmonella typhi</i>	24	35	52	-
<i>Shigella dysenteriae</i>	22	32	50	-
<i>Trichophytum rubrum</i>	0	0	-	27
<i>Candida albicans</i>	0	0	-	44
<i>Microsporan spp</i>	0	0	-	30

**Table 3.** The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of petroleum ether and methanolic extracts of *G. aqualla* against test organisms.

Microorganism	Petroleum ether extract		Methanolic extract	
	MIC(mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)
<i>Staphylococcus aureus</i>	6.25	25.00	1.25	2.50
<i>Enterococcus aerogenes</i>	6.25	25.00	1.25	5.00
<i>Escherichia coli</i>	3.13	12.50	2.25	5.00
<i>Salmonella typhi</i>	6.25	25.00	1.25	2.50
<i>Shigella dysenteriae</i>	6.25	25.00	1.25	2.50

therefore gives scientific basis and credence for the claims of the therapeutic capabilities and folkloric usage of the stem bark of *G. aqualla* for the treatment of various ailments.

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## Conflict of interests

The author(s) have not declared any conflict of interests.

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