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PHYTOCHEMICAL SCREENING AND *IN VITRO* ANTIMICROBIAL ACTIVITY OF METHANOLIC ROOT EXTRACT OF *Ampelocissus grantii* (Baker) Blanch. (*Vitaceae*)

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ABSTRACT

A. grantii is a medicinal plant widely used in traditional medicine for the treatment of many diseases including bacterial and fungal infections. This research was designed to conduct phytochemical screening and investigate the antimicrobial potential of methanolic extract of *A. grantii* root. The sample was collected, shade dried, ground into powder and extracted by maceration using 100 % methanol. The liquid extract was evaporated to dryness and subjected to phytochemical screening and antimicrobial test using various concentrations (200, 100, 50 and 25 mg/ml) to examine the susceptibility of clinical isolates of bacteria (*Pseudomonas aeruginosa, Escherichia coli*) and fungi (*Aspergillus niger* and *Candida albicans*) using standard procedures. Phytochemical screening revealed the presence of steroid/triterpenes, tannins, flavonoids, saponins and proteins. However, alkaloids were not detected. The result showed that the activity of the extract is concentration dependent, with the highest value of the zone of inhibition observed at 200 mm/ml for both antibacterial and antifungal tests. The antimicrobial activity may be due to the presence of these phytochemicals. Therefore, *A. grantii* can be used a precursor for new antimicrobial drug synthesis.

Keywords: Antibacterial activity, Antifungal Activity, *Ampelocissus grantii*, Phytochemical Screening, Maceration.

INTRODUCTION

Diarrhea is one of the main water-borne diseases that is widespread throughout the world and is the leading cause of death for children under the age of five (Laloo and Hemalatha, 2011). Diarrhea claimed the lives of up to 526,000 children worldwide in 2015, out of which 77,000 were Nigerian. Most occurrences of diarrhea are brought on by rotavirus, *Escherichia coli* (WHO, 2019), *Staphylococcus aureus*, and the fungus *Candida albicans* in low-resource Nations (Maiyama *et al.,* 2023). It is now more common for patients who are getting antifungal prophylaxis or need long-term treatment to acquire resistance to antifungal medications. According to reports, the most common fungi include Pneumocystis, Candida, Aspergillus and Cryptococcus species and cause up to 1.4 million fatalities globally per annum (Brown *et al*., 2012), mostly in those with compromised immune systems (HIV/AIDS patients). In 2013, Oladele *et al.* revealed that 1.5 million of the 38 million Nigerian women aged between 15 and 50 years get recurrent vaginal thrush. Over 15.5 million children, according to local

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estimates, have Tinea capitis, which is estimated to affect over 20% of school-age children (Tsafe *et al*., 2019).

In addition to being an essential source of therapeutic medications, plants are crucial to the survival of tribal and ethnic societies. 20000 plant species from various families are thought to be suitable for these purpose (Zongo *et al.,* 2010). In addition, they are a key source of pharmaceuticals and offer bioactive molecules that can be used either as synthetic analogs or as drugs themselves (Maiyama *et al.,* 2023). Due to their safety, affordability, and effectiveness, plants are used to treat ailments by almost 80% of the world's population (Handral *et al.,* 2012).

In an effort to widen the range of antimicrobial agents from natural sources, *Ampelocissus grantii* (also known as "Rongon daji" and "eteku" in Hausa and Yoruba languages respectively), which belongs to the family Vitaceae, was chosen for this research. For the treatment of diarrhoea (Etuk *et al.,* 2009), cancer (Muhammad and Amusa, 2005), muscular pain and trypanosomiasis, the plant is available and used in the community (Bizimana *et al*., 2006). In the Philippines, methanol extracts of this plant yielded several bioactive chemicals that showed inhibitory activity of cancer cell development against some human cancer cell lines (Pettit *et al*., 2008). Terpenoids, flavonoids, tannins, and saponins were found to be present in methanol extracts of the aerial portions of *A. grantii* (Baker) Planch (Musa*, et al*., 2010). According to Yahaya (2016), the plant's aerial portions also exhibit a broad variety of antimicrobial action against yeast (*Candida albicans*), gram negative (*E. coli, Klebsiella pneumonia, Pseudomonas aeruginosa*) and gram positive bacteria (*Staphylococcus aureus, Bacillus subtilis*). This research is aimed at conducting phytochemical screening and *in vitro* antimicrobial activity of the crude methanol extract of *Ampelocissus grantii* root. The objectives are to extract the dried, powdered root sample with methanol, conduct preliminary phytochemical screening and investigate the antibacterial and antifungal potential of the extract.

MATERIALS AND METHODS

Collection of Plant Material

The root of *A. grantii* was collected from Tambuwal town in Tambuwal Local Government Area of Sokoto State, Nigeria. The plant was authenticated at the Herbarium Section of the Department of Biological Sciences, Usmanu Danfodiyo University Sokoto, Nigeria and was given voucher number (UDUH/ANS/0159). The root was air dried under shade, ground into fine powder using porcelain mortar and pestle and stored in polyethylene plastic bag until use.

Extraction of the Sample

The extraction was carried out using the method of Maiyama *et al.,* (2020). 100 g of the sample was extracted with methanol (1: 5 w/v) in a closed container by maceration with occasional shaking for four days. The mixture was decanted and the residue was extracted three more times using fresh solvent (methanol). The extract obtained was filtered with muslin cloth, Whatmann no. 1 filter paper and concentrated on water bath at 45 ^{0}C. The dried extract was stored in a container and labeled for further analysis.

Phytochemical Screening

The extract was screened for the presence of steroids/triterpenes, alkaloids, tannins, flavonoids, saponins, and proteins as described by Evans (2006) and Sofowora (2008)

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Antimicrobial Studies

Test Organisms

The organisms used in this research work were *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans* and *Aspergillus niger* obtained from the Department of Veterinary Microbiology, Usmanu Danfodiyo University Sokoto. All the bacterial cultures were checked, grown on Muller Hinton Agar slant while the fungi were grown on a slant of Sabraud Dextrose Agar (SDA).

Microbial Growth Media Preparation

The manufacturer's instructions for preparing culture media were followed. 38 grams of Muller Hinton agar was weighed, combined with 1000 cm^3 of distilled water, and autoclaved at 120 ^{0}C for 20 minutes under 1 bar of pressure to sterilize it. The medium was then dispensed into petri dishes to produce a uniform depth. The culture media was allowed to cool at room temperature without being disturbed until it solidified. Then, it was sterilized for 24 hours in an inverted manner in an incubator at 37 ^{0}C. Then, plastic bags were used to preserve the prepared plates between 4 and 8 degrees Celsius. Mueller Hinton Agar was used for *Escherichia coli*, and *Pseudomonas aeruginosa* while Saboraud Dextrose Agar was used for *Candida albicans* and *Aspergillus niger.*

Determination of Antibacterial Activity

Here 0.1cm^3 of the respective standardized inoculums (0.5 Mcfarland turbidity standard = 1.0x10^8cfu/cm^3) of each test bacteria were spread into sterile Mueller Hinton Agar plates so as to achieve even growth. A sterile cork borer (8.0 mm in diameter) was used to aseptically drill wells into the Agar plates after the plates had dried. Using 10% dimethylsulfoxide (DMSO), the extract was sequentially diluted to achieve various concentrations of 25, 50, 100, and 200 mg/cm^3. Then, 200 microliters of each concentration were added to the wells that had been previously drilled in the agar plates. Diameter of zones of inhibition developed around the wells showed the degree of susceptibility of the organisms to the extract. With the aid of a transparent ruler, the zones of inhibition were measured (Lino and Deogracous, 2006).

Determination of Antifungal Activity

The standard inoculum (0.1 mL, 0.5 McFarland turbidity standard = 1.0 x 108 cfu/mL) of each test fungus was spread into two sterile Saboraud Dextrose Agar plates so as to achieve even growth. The plate was allowed to dry and a sterile cork borer (8.0 mm diameter) was used to bore holes aseptically in the agar plates. The extract was prepared and serially diluted using 10% dimethylsulphoxide (DMSO) to achieve different concentrations of 25, 50, 100 and 200 mg/mL. Subsequently, 200 µL of each concentration of the extracts was introduced into the bored wells. At 37 °C for 48 hours, the extract was allowed to diffuse into the medium in an incubator. As a positive control, 5 mg/mL was prepared and poured into the single hole in the middle of one of the petri dishes. Antifungal activity of the extracts was determined by measurement of zones of inhibition produced around the wells. The test fungi's level of susceptibility was evaluated by the zones' diameter (Lino and Deogracious, 2006; Tsafe *et al.,* 2019).

RESULTS AND DISCUSSION

Phytochemical Screening

Preliminary phytochemical screening of the methanolic root extract of *A. grantii* revealed the presence of steroids/triterpenes, tannins, flavonoids, saponin and protein. The result is presented in Table 1.

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Table 1: Result of phytochemical screening

Test	Present/absent
Steroids/Triterpenes	+
Alkaloids	-
Flavonoids	+
Saponins	+
Tannins	+
Proteins	+

Key: + = Present, - = Absent

Antibacterial Activity

The result of antibacterial activity of the methanolic root extract of *A. grantii* against clinical isolates of *Escherichia coli* and *Pseudomonas aeruginosa* is presented in Table 2.

Table 2: Result of antibacterial activity

Conc. (mg/ml)	Diameter of Zones of Inhibition (mm)	
	Escherichia coli	*Psuedomonas aeruginosa*
200	22	23
100	17	19
50	15	16
25	13	13

Antifungal Activity

Table 3 shows the result of antifungal susceptibility test of the methanolic root extract of *A. grantii* against clinical isolates of *C. albicans* and *A. niger.*

Table 3: Result of antifungal activity

Conc. (mg/ml)	Diameter of Zones of Inhibition (mm)	
	Aspergillus niger	*Candida albicans*
200	26	21
100	22	19
50	19	15
25	14	12
Fluconazole (5 mm/mL)	12	15

From Table 1, the result of the phytochemical analysis of the root crude methanol extract showed the presence of steroids, triterpenes, tannins, flavonoids, saponins, and proteins. Zongo *et al.* (2010) reported the presence of flavonoids, saponins, steroids, and triterpenes in *A. grantii*. The current research, however, revealed the absence of alkaloids. It was reported that Vitaceae family has 14 genera and 800 described species but only few of them contain significant alkaloids (Zonzo *et al.,* 2010). Absence of alkaloids in this plant agrees with the report of Tijjani *et al.,* (2012), Zongo *et al.,* (2010), Etuk *et al*., (2009) and Maiyama *et al.* (2023). The antimicrobial action that has been observed and the extra use of *A. grantii* in traditional medicine could both be attributed to the presence of these metabolites in the plant's root. This is due to the scientific evidence that these secondary metabolites function as antioxidants, anti-inflammatories, anticancer agents, and antibacterial agents (Singh *et al*., 2002; Stapleton *et al.,* 2004, 2007; Maiyama *et al.,* 2023). Furthermore, among the different phytochemicals, phenolic compounds such as flavonoids and tannins have gained attention of different areas of applications such as pharmaceutical, health, food, and cosmetic industries. These compounds are widespread in the plant kingdom as part of our daily diet and are attractive as natural antioxidants (Jaradat *et al.,* 2016).

The extensive use of profit-oriented antimicrobial medications has led to several drug resistant strains (Motamedi *et al.,* 2010). Beyond resistance, some synthetic antibiotics are showing unwanted side effects (Zonzo *et al.,* 2010). This have led to the screening for more effective, less toxic and cost effective drugs from natural sources (Bhatt and Neggi, 2012). The use of plant extracts for therapeutic purposes and for their antibacterial, antifungal, and antiviral activities is widespread around the world. In this study, the antibacterial effects of crude methanol extract of *Ampelocissus grantii* root against *E. coli* and *P. aeruginosa* were examined. Similarly, the antifungal activity

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was checked against *C. albicans* and *A. niger*. When the diameter of the inhibition zone (DIZ) detected is greater than or equal to 9 mm around the holes, it is possible that there is antimicrobial activity (Kitzberger *et al.,* 2007; Zonzo *et al.,* 2010; Maiyama *et al.,* 2023).

The bacterial test organisms showed susceptibility at all concentrations with *P. aeruginosa* showing slightly more sensitivity than *E. coli* (Table 2). The findings indicated that the concentration of an extract determines its activity (the higher the concentration the greater the activity). Similar outcome was reported by El-Mahmood *et al.* (2008) and Maiyama *et al.* (2020). According to the results of the antibacterial properties mentioned above, the extracts contain a secondary metabolite that can stop the growth of some microorganisms. The presence of components like tannins and saponins, which have been demonstrated to have antimicrobial characteristics (Ragasa *et al*., 2005), is what gives the extracts their antibacterial property. These components are thought to work by disrupting the bacterial cell membrane (Hendrich, 2006). The extract's large zone of inhibition against *P. aeruginosa* suggests that it can be used to treat sores and open wounds, and their activity against *E. coli* suggests that it can be used to treat diarrhea and dysentery (Brooks *et al*., 2002).

The growth of both *A. niger* and *C. albicans* were inhibited by the crude methanol extract of *A. grantti* root (Table 3) at all concentrations (200 mg/ml, 100 mg/ml, 50 mg/ml, and 25 mg/ml). With diameter of inhibition zone of 26 mm at 200 mg/mL, *A. niger* is more sensitive to the extract compared to 21 mm observed at the same concentration for *C. albicans*. The outcome further demonstrated that the extract's action is concentration-dependent (as in the case of antibacterial test). Several researchers, including El-Mahmood *et al*. (2008), Kamoldeen and Fola (2015), Tsafe *et al*. (2019), and Maiyama *et al*. (2020), have reported similar findings. With inhibitory diameter of 15 mm against *C. albicans* and 12 mm against *A. niger* at just 5 mg/ml, the conventional drug (fluconazole) demonstrated antifungal action. When compared to the extract, the standard recorded higher activity. This is due to the antifungal drug's unadulterated nature (Prescott *et al.,* 2002; Tsafe *et al.,* 2019). The presence of phytochemicals like triterpenes may be the cause of the observed antifungal (Maiyama *et al.,* 2020) and antibacterial (Arora *et al.,* 2017) activity. The antimicrobial activity may also be as a result of the steroids in the plant (Arora *et al.,* 2017).

CONCLUSION

The root crude methanol extract of *A. grantii* obtained by maceration showed the presence of steroids, triterpenes, tannins, flavonoids, saponins and proteins. The antimicrobial studies conducted using agar well diffusion method revealed that the extract is active against clinical isolates of *Psuedomonas aeruginosa, Escherichia coli, Aspergillus niger* and *Candida albicans.* The test organisms are most susceptible at highest prepared concentration. This study justified the plant's use in traditional medicine for the treatment of microbial diseases.

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