



Research Article

Phytochemical Screening and *In Vitro* Antimicrobial Activity of the Root Extracts of *Ampelocissus grantii* (Baker) Blanch. (Vitaceae)

*¹Maiyama, S. Y., ²Dahiru, H., ²Mohammed, M. L., ³Halilu, M. E., ⁴Ugwah-Oguejiofor, C. J. and ⁵Babagana, A

¹Department of Chemistry, Air Force Institute of Technology, Kaduna, Nigeria

²Department of Pure and Applied Chemistry, Usmanu Danfodiyo University Sokoto, Nigeria

³Department of Pharmacognosy and Ethnopharmacy, Usmanu Danfodiyo University Sokoto, Nigeria

⁴Department of Pharmacology, Usmanu Danfodiyo University Sokoto, Nigeria

⁵Department of Chemistry, Borno State University, Maiduguri, Nigeria

*Corresponding author: salmanmaiayama@gmail.com, doi.org/10.55639/607.1827

ARTICLE INFO:

ABSTRACT

Keywords:

Ampelocissus grantii,
Phytochemical screening,
antibacterial activity,
antifungal activity
Escherichia coli

The medicinal plant *Ampelocissus grantii* is frequently used in traditional medicine to treat a variety of illnesses, including bacterial, parasite, and fungal infections. This study was created to look into the antibacterial properties and do a preliminary phytochemical screening of extracts from *A. grantii* roots. The root sample of *A. grantii* was ground into powder and extracted successively by maceration using n-hexane, ethyl acetate and methanol. The antimicrobial profile of various concentrations (200mm/ml, 100 mm/ml, 50 mm/ml and 25 mm/ml) of the extracts was examined against clinical isolates of bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*) and fungi (*Aspergillus niger* and *Candida albicans*) using standard procedures. Preliminary phytochemical screening of the extracts was conducted to test for the presence of steroid/triterpenes, alkaloids tannins, flavonoids, saponins and proteins. The highest antimicrobial activity was observed in methanol extract, followed by ethyl acetate extract with *E. coli* and *A. niger* being the most susceptible. The result also showed that the activity of all the extracts is concentration dependent, with the highest value of the zone of inhibition observed at 200 mm/ml. Preliminary phytochemical screening revealed the presence of steroid/triterpenes, tannins, flavonoids, saponins and proteins. However, alkaloids were not detected in any of the extracts. The antimicrobial activity may be due to the presence of these phytochemicals. Therefore, *A. grantii* can be used to source new drugs for the treatment of various microbial infections.

Corresponding author: Maiyama, S. Y. Email: salmanmaiayama@gmail.com
Department of Chemistry, Air Force Institute of Technology, Kaduna, Nigeria

INTRODUCTION

According to Lola and Hemalatha (2011), diarrhoea 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. In 2015, diarrhoea claimed the lives of up to 526,000 children worldwide, 77,000 of whom were Nigerian. Most occurrences of diarrhoea are brought on by rotavirus, *Escherichia coli* (WHO, 2019), *Staphylococcus aureus*, and the fungus *Candida albicans* in low-resource environments (Anne and Geboes, 2002). It is now more common for patients who need long-term treatment or who are getting antifungal prophylaxis to acquire resistance to antifungal medications. According to reports, the most common fungi include *Candida*, *Aspergillus*, *Pneumocystis*, and *Cryptococcus* species and cause up to 1.4 million fatalities globally each year (Brown *et al.*, 2012), most frequently in those with compromised immune systems (HIV/AIDS patients). According to Oladele *et al.* (2013), there are 38 million women in Nigeria between the ages of 15 and 50, making up 50% of the country's population of over 155 million. Recurrent vaginal thrush affects 1.5 million Nigerian women. Over 15.5 million children, according to local 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 objectives (Zongo *et al.*, 2010). In addition, they are a key source of pharmaceuticals and offer bioactive molecules that can be used either as synthetic analogues or as drugs themselves (Cragg and Newman, 1999). 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). To widen the range of antimicrobial agents from natural sources, *Ampelocissus grantii* (also known as “Rongon daji” or “Tanbi” in the Hausa language and “eteku” in the Yoruba language), which belongs to the Vitaceae family, was chosen for this research. For the treatment of diarrhoea (Etuk *et al.*, 2009), cancer (Muhammad and Musa, 2005), muscular pain, and trypanosomiasis, the plant is available and used in the community (Adjanohoun *et al.*, 1980; Bizimana *et al.*, 2006). Both *in vivo* and *in vitro* tests of the plant's trypanocidal efficacy were positive (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 have all been 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 antibacterial action against the 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 root extracts of *Ampelocissus grantii*. The objectives are to extract the sample successively by maceration using three solvents (n-hexane, ethyl acetate and methanol), investigate the antimicrobial potential and conduct preliminary phytochemical screening of the extracts.

MATERIALS AND METHODS

Sampling and Preparation of Plant Material

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

Extraction

The extraction was carried out using the method of Tsafe *et al.*, (2019) with slight modification. The root sample of the *Ampelocissus grantii* was ground into powder and extracted with n-hexane (1: 5 w/v) in a closed container by cold extraction (maceration) with occasional shaking for five days. The extract obtained was filtered with muslin cloth, Whatmann no. 1 filter paper and concentrated on water bath at 45 °C after which the extract was air dried. The residue was macerated with ethyl acetate and then methanol respectively using the same procedure as described above. All the extracts obtained after drying were stored in different containers and labeled for further analysis.

Preliminary Phytochemical Screening

The extracts were screened for the presence of steroids/triterpenes, alkaloids, tannins, flavonoids, saponins, and proteins as described by Evans (2006).

Test Organisms

The organisms used in this research work were *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans* and *Aspergillus niger*. They were all 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).

Preparation of the Media

The manufacturer's instructions for preparing culture media were followed. 38 grams of Muller Hinton agar was weighed, combined with 1000 cm³ of distilled water, and autoclaved at 120 °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 °C. Then, plastic bags were used to preserve the prepared plates between 4 and 8 degrees Celsius.

Determination of Antibacterial Activity

Here 0.1cm³ of the respective standardized inoculums (0.5 Mcfarland turbidity standard = 1.0x10⁸cfu/cm³) 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 extracts were sequentially diluted to achieve various concentrations of 25, 50, 100, and 200 mg/cm³ for each extract, respectively. Then, 200 microliters of each concentration of the prepared extract were added to the wells that had been previously drilled in the Agar plates. Zones of inhibition developed around the wells show that there is activity. 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 sterile Sabraud Dextrose Agar plates so as to achieve even growth. The plates were allowed to dry and a sterile cork borer (8.0 mm

diameter) was used to bore wells aseptically in the agar plates. The extracts were prepared and serially diluted using 10% dimethylsulphoxide (DMSO) to achieve different concentrations of 25, 50, 100 and 200 mg/mL respectively for each extract. Subsequently, 200 µL of each concentration of the extracts was introduced into the wells earlier bored in the Agar plates. At 37 °C for 48 hours, the extracts were allowed to diffuse into the medium (kept for 1 hour on the bench prior to incubation). As a positive control, fluconazole (5 mg/mL) was made by

combining 50 mg of the drug powder with 10 cm³ of DMSO. This solution was then poured into the single hole in the middle of two petri dishes containing *C. albicans* and *A. niger*, respectively. Antifungal activity of the extracts was determined by measurement of zones of inhibition produced around the wells (meter rule was placed in 3 different directions and average was calculated). The test fungi's level of susceptibility was evaluated by the zones' diameter (Lino and Deogracious, 2006; Tsafe *et al.*, 2019).

RESULTS AND DISCUSSION

Table 1: Result of antibacterial activity

Conc. (mg/ml)	Diameter of Zones of Inhibition (mm)					
	ME		EE		HE	
	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>E. coli</i>	<i>P. aeruginosa</i>
200	30	20	20	13	10	11
100	26	18	10	R	R	10
50	20	14	R	R	R	R
25	14	12	R	R	R	R

Key: *E.coli* = *Escherichia coli*, *P. aeruginosa* = *Psuedomonas aeruginosa*, R= Resistant
ME= methanol extract, EE= ethyl acetate extract, HE= hexane extract

Table 2: Result of antifungal activity

Conc. (mg/ml)	Diameter of Zone of Inhibition (mm)					
	ME		EE		HE	
	<i>A. niger</i>	<i>C. albicans</i>	<i>A. niger</i>	<i>C. albicans</i>	<i>A. niger</i>	<i>C. albicans</i>
200	24	20	18	21	10	R
100	20	17	14	18	R	R
50	15	15	11	13	R	R
25	12	10	R	11	R	R
Fluconazole (5 mg/ml)	12	15	12	15	12	15

Key: *A. niger*= *Aspergillus niger*, *C. albicans*= *Candida albicans*, R= Resistant
ME= methanol extract, EE= ethyl acetate extract, HE= hexane extract

Table 3: Result of phytochemical screening

Test	HE	EE	ME
Steroids/Triterpenes	+	+	+
Alkaloids	-	-	-
Flavonoids	-	+	+
Saponins	-	-	+
Tannins	-	+	+
Proteins	-	-	+

Key: ME= methanol extract, EE= ethyl acetate extract, HE= hexane extract

+ = Present, - = Absent

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 *Ampelocissus grantii* root extracts against *E. coli* and *Pseudomonas aeruginosa* were examined. When the diameter of the inhibition zone (DIZ) detected is 9 mm or more around the holes, it is possible that there is antimicrobial activity (Kitzberger *et al.*, 2007). The methanol extract had the maximum inhibitory activity against the two test species, followed by the ethyl acetate extract, while the n-hexane extract had the lowest inhibitory activity against *E. coli* and *P. aeruginosa* (Table 1). At a dosage of 200 mg/ml, all of the extracts were discovered to be effective against *P. aeruginosa* and *E. coli*. At doses of 50 mg/ml and 25 mg/ml, respectively, both organisms displayed resistance to ethyl acetate and n-hexane extracts. The findings indicated that the concentration of an extract determines its activity (the higher the concentration the greater the activity). El-Mahmood *et al.* (2008) had reported a similar outcome. According to the results of the antibacterial properties mentioned above, the extracts contain a secondary metabolite that can prevent some microorganisms from growing. 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 extracts' wide zone of inhibition against *Pseudomonas aeruginosa* suggests they can be used to treat sores and open wounds, and their activity against *Escherichia coli* suggests they can be used to treat diarrhea and dysentery (Brooks *et al.*, 2002).

Aspergillus niger and *Candida albicans* were indeed inhibited by the extracts of *Ampelocissus grantii* root (Table 2). At all concentrations (200 mg/ml, 100 mg/ml, 50 mg/ml, and 25 mg/ml), methanol and ethyl acetate extracts demonstrated antifungal activity against the test organisms (*Aspergillus niger* and *Candida albicans*), whereas n-hexane extract demonstrated antifungal activity against only *Aspergillus niger* at the highest concentration (200 mg/ml). On the examined fungi, methanol extract showed the greatest activity, followed by ethyl acetate, while n-hexane extract showed the least amount of activity. The outcome further demonstrated that the extracts' 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 an average 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 extracts, the standard recorded the highest 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 activity that was observed (Maiyama *et al.*, 2020).

According to Table 3, the findings of the phytochemical analysis of the root of *Ampelocissus grantii* showed the presence of steroids, triterpenes, tannins, flavonoids, saponins, and proteins. Zongo *et al.* (2010) reported the presence of these flavonoids, saponins, steroids, and triterpenes in *A. grantii*. All the extracts demonstrated the presence of triterpenes and steroids. Additionally, the n-hexane extract contained only steroid and triterpene compounds. However, only the methanol extract showed signs of proteins and saponins. None of the extracts contained alkaloids, which was in agreement with the report of Tijjani *et*

al., (2012), Zongo *et al.*, (2010), and Etuk *et al.*, (2009). The antibacterial action that has been observed and the extra use of *Ampelocissus 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).

CONCLUSION

The root extracts of *Ampelocissus grantii* obtained from successive extraction by maceration using n-hexane, ethyl acetate and methanol showed the presence of steroids, triterpenes, tannins, flavonoids, saponins and proteins. The antimicrobial studies conducted using agar well diffusion method revealed that the extracts are active against clinical isolates of *Psuedomonas aeruginosa*, *Escherichia coli*, *Aspergillus niger* and *Candida albicans*. The test organisms are most susceptible to methanol extract and least to n-hexane extract. This study justified the plant's use in traditional medicine for the treatment microbial diseases.

REFERENCES

- Adjanohoun, E. Ahyi, M.R.A., Ake ssi, L., Dan Dicko, L., Daouda, (1980). Contribution to Ethnobotany and Floristic Studies Niger Acct. Paris ISBN 929025009-3 pp. 250.
- Anne, J.M, Geboes, K. (2002). Infectious colitis. *Acta Endoscopica* 3:2.
- Bizimana, N., Tietjen, U., Dessin, K. H., Diallo, D., Djibril, C., Melzig, M. F., and Clausen, P. H. (2006). Evaluation of Medicinal Plants from Mali for their in vitro and in vivo Trypanocidal Activity. *Journal of Ethnopharmacology*, 105:385-399.
- Brooks, G.F., Butel, J.S., Morse, S.A. (2002). *Jawetz, Melnick and Andelberg's medicinal microbiology (2nd Edition)*. McGraw-Hill, New Delhi, India, pp. 197,234,550.
- Brown, G. D., Denning, D. W., Gow, N. A. R., Levitz, S. M., Netea, M. G., White, T. C., (2012). Hidden killers: human fungal infections. *Science Translational Medicine* 4, 165 rv113.
- Cragg, G. M. and Newman, D. J. (2005). International Collaboration in Drug Discovery and Development from Natural Sources. *Pure Applied Chemistry*, 77 (11): 1923–1942.
- El-Mahmood, A. M., Doughari J. H. and Ladan, N. (2008). Antimicrobial screening of stem bark extract of *Vitellaria paradoxa* against some enteric pathogenic microorganisms. *African Journal of Pharmacy and Pharmacology*. 2(5): 089-094.
- Etuk, E.U., Ugwah, M.O., Ajagbonna. O.P., Onyeyilli, P.A. (2009). Ethnobotanical

- survey and preliminary evaluation of medicinal plants with antidiarrhoea properties in Sokoto State, Nigeria. *Journal of Medicinal Plant Research* 3(10): 763 – 766.
- Evans, W.C. (2006). *Trease and Evans Pharmacognosy, 15th ed.*, Saunders an Imprint of Elsevier. India. Pp. 223-308.
- Handrail, H. K., Pandith, A. and Shruthi S. D. 2012. A review of *Murraya koenigii*: multipotential medicinal plant. *Asian Journal pharmaceutical and clinical research* 5(4): 5-14
- Hendrich, A.B. (2006). Flavonoid Membrane Interaction, Possible Consequences for Biological Effects of some Polyphenolic Compounds. *Acta Pharmacologica Sinica* 27(1): 27–40.
- Inngjerdingen, K. Nergard, C. S., Diallo, D., Mounkoro, P. P. and Paulson, B. S. (2004). An ethnopharmacological survey of plants used for wounds healing in Dogonland Mali, West Africa. *Journal of Ethnopharmacology* 92: 233 – 244.
- Kitzberger, C. S. G., Smania, A., Pedrosa, R. C. and Ferreira, S. R. S. (2007). Antioxidant and antimicrobial activities of Shiitake (*Lentinula edodes*) extracts obtained by organic solvents and super critical fluids. *Journal Food Engineering*, 80 (2): 631-638.
- Laloo, D. and Hemalatha, S. (2011). Ethnomedicinal Plants used for diarrhea by tribals of Meghalaya, Northeast India. *Pharmacognosy Reviews* 5: 147 – 154.
- Lino, A. and Deogracious, O. (2006). The in vitro antibacterial activity of *Annona senegalensis*, *Securidacalongi pendiculata* and *Steganotae niaaraliacea*. Ugandan Medicinal Plants. *Journal of African Health Sciences*, 6 (1): 31- 35.
- Loeffler, J. and Stevens, D. A. (2013). Antifungal Drug Resistance. *Clinical Infectious Diseases*, 36 (1): 31-41.
- Maiyama, S. Y., Tsafe, A. I., Dabai, M. U., Halilu, M. E., Bello, S. S., Dahiru, H., Jiya, A. H., Danjuma, A. M., Babagana, A. and Abubakar, H. (2020). Isolation and Characterization of Triterpene from n-hexane Stem Bark Extract of *Vitellaria paradoxa* (Shea Butter Tree). *Caliphate Journal of Science and Technology*, 2: 167-174.
- Muhammad, S., Amusa, N.A. (2005). The important food crops and medicinal plants of North-Western Nigeria. *Research Journal of Agriculture and Biological Sciences*. 1(3): 254 – 260
- Musa, Y. M., Sule, M. I., Pateh, U. U., Ilyas, M., Musa, A. M., Usman, H and Abdullahi, M.S. (2010). Preliminary phytochemical and antimicrobial studies of methanol extract of the aerial parts of *Ampelocissus grantii* (Baker) Planch. *International Journal of Chemical Sciences*, 3(2):167-174.
- Oladele, R. and Denning D. W. (2014) Burden of serious fungal infections in Nigeria, *West African Journal of Medicine*, 33 (2): 107-114.
- Pettit, G. R., Mukku, J. V., Craft, G., Herald, D. L. and Knight, J. C. (2008). Antineoplastic. 558. *Ampelocissus* sp. Cancer cell inhibitory constituents. *Journal of Natural Products*, 71: 130-133
- Prescott, M. L., Harley, P. J. and Klein, A. D. (2002). *Microbiology*. 7th edition. McGraw Hill Inc.
- Ragasa, C.Y. Deluna, R.D., Hofilena, J.G. (2005). Antimicrobial terpenoids from *Pterocarpus indicus*. *Natural Products Research*. 19(4): 305 – 309.
- Singh, R.P., Dhanalakshimi, S. and Agarwal, R. (2002). Phytochemicals as cell cycle modulators, a less toxic approach in halting human cancers. *Cell cycle* 1:155 – 160.
- Stapleton, P.D., Shah, S., Anderson, J.C., Hara Y. and Taylor, P.W. (2004). Modulation of beta – lactam resistance in *phylococcusaureris* by catechins and gallates, *International Journal of Antimicrobial Agents*, 23:462 – 467.

- Stapleton, P.D., Shah, S., Ehlert, K. Hara, Y. and Taylor P.W. (2007). The beta-lactam resistance modifier (-) epicatechingallates alter the architecture of the cell wall of staphylococcus aureus. *Microbiology*, 153:2093 – 2103.
- Tijjani, A., Ndukwe, I.G. and Ayo, R.G. (2012). Isolation and Characterization of Lup 20(29)-ene-3, 28- diol (Betulin) from the Stem Bark of *Adenium obesum* (Apocynaceae). *Tropical Journal Pharmaceutical Research*, **11** (2): 259.
- Tsafe, A. I., Dabai, M. U., Halilu, M. E., Maiyama, Y. S., Bello, S. S., Dahiru, H., and Jiya, A. H. (2019). Comparative Phytochemical and Antifungal Activities of Stem and Root Bark Extracts of *Vitellaria paradoxa* (Shea Butter) Tree. *Caliphate Journal of Science and Technology*, 1: 1-5.
- World Health Organization. (2019). One is Too Many: Ending child deaths from pneumonia and diarrhea. 1-40
- Yahaya, M. M. (2016). Phytochemical and some Biological Studies of the Methanol Extract of the Aerial Parts of *Ampelocissus grantii* (Baker) Planch. (Vitaceae). *Doctor of Philosophy Thesis*. p 162
- Zonzo, C., Savadogo, A., Ouattara, L., Bassole, I. H. N., Ouattara, C. A. T., Ouattara, A. S., Barro, N., Koudou, J. and Traore, A. S. (2010). Polyphenols Content, Antioxidant and Antimicrobial Activities of *Ampelocissus grantii* (Baker) Blanch. (Vitaceae): A Medicinal Plant from Bukina Faso. *International Journal of Pharmacology*, 6(6): 880-887.