Journal of Applied Sciences

ISSN 1812-5654

science alert

ANSInet
an open access publisher
http://ansinet.com

OPEN ACCESS Journal of Applied Sciences

ISSN 1812-5654
DOI: 10.3923/jas.2019.400.405

Research Article
Anti-bacterial Efficacy of *Mitracarpus villosus* Extract on Some Selected Multi-drug-resistant Clinical Isolates

Ibrahim Sani, Abubakar Abdulhamid, Fatima Bello, Isah Musa Fakai and Aishatu Sa'idu

Department of Biochemistry, Kebbi State University of Science and Technology, Aliero, Nigeria

Abstract

Background and Objective: Anti-biotic resistance has been increasing in prevalence, thus becoming a major medical challenge worldwide and posing a big threat to human society. Hence, it becomes essential to search for newer drugs with lesser rate of resistance development and lesser toxicity especially from plant resources. **Materials and Methods:** *Mitracarpus villosus* plant was obtained within Aliero, Nigeria, while the multi-drug-resistant bacteria (*Staphylococcus aureus*, *Klebsilla pneumoniae*, *Streptococcus pneumoniae and Salmonella typhi*) used were obtained from Sir Yahaya Memorial Hospital Birnin Kebbi, Nigeria. Acetone extract of the plant was used and test organisms were taken from nutrient agar slants and sub-cultured on nutrient agar plates. The anti-bacterial activities were conducted using agar well diffusion method. **Results:** The plant phytochemical composition shows the presence of saponins, tannins, alkaloids, terpenoids, steroids, anthraquinones, flavonoids and phenols. The result for the anti-bacterial activity reveals that, the zones of inhibition at extract concentration of 120 mg mL^{-1} were 5.89±0.38 mm for *S. aureus*, 5.22±0.19 mm for *S. pneumoniae*, 6.89±0.70 mm for *K. pneumoniae* and 6.11±0.70 mm for *Salmonella typhi*. The higher the concentration of the plant extract the higher the activity and at lower concentration, the activity reduces significantly. **Conclusion:** The results obtained revealed that, *M. villosus* has anti-bacterial activity against the tested multi-drug-resistant micro-organisms, which was attributed to the presence of some of the phytochemicals in the plant extract.

Key words: Anti-bacterial, Anti-biotic resistance, multi-drug-resistance, *Mitracarpus villosus*, clinical isolates

Citation: Ibrahim Sani, Abubakar Abdulhamid, Fatima Bello, Isah Musa Fakai and Aishatu Sa'idu, 2019. Anti-bacterial efficacy of *Mitracarpus villosus* extract on some selected multi-drug-resistant clinical isolates. J. Applied Sci., 19: 400-405.

Correspo nding Author: Ibrahim Sani, Department of Biochemistry, Kebbi State University of Science and Technology, Aliero, Nigeria Tel: +2348037731851

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Anti-biotics are chemical substance which serves as agents to kill or inhibit the growth of micro-organisms[1]. For many years, anti-biotics have been used to treat many bacterial diseases. One of the central themes of success in human therapeutics in the 20th century was the discovery and development of anti-biotics and anti-bacterial agents. However, the usage of anti-biotics and anti-bacterial chemotherapeutics is becoming more restricted, because bacteria are capable of developing resistance soon after their introduction[2] and most anti-biotics have side effects[3]. Over the years, there is a decrease in microbial susceptibility to exiting anti-microbial agents, responsible for drug resistance which is exerting global problems today. In fact the theme of World Health Day 2011 was "no action today no cure tomorrow". Antibiotic resistance has been increasing in prevalence[4]. The extraordinary genetic capacities of microbes have benefitted from man's overuse of antibiotics to exploit every source of resistance genes and every means of horizontal gene transmission to develop multiple mechanisms of resistance for each and every antibiotic introduced into practice clinically, agriculturally or otherwise[5]. The successful use of any therapeutic agent is compromised by the potential development of tolerance or resistance to that compound from the time it is first employed[6].

Anti-biotic discovery, modes of action and mechanisms of resistance have been productive research topics in academia[7] until recently, in the pharmaceutical industry. As natural products, they provide challenging intellectual exercises and surprises with respect to their chemical nature, biosynthetic pathways, evolution and biochemical mode of action[8].

To overcome the problem of resistance of anti-biotic, medicinal plants have been exclusively studied as alternative treatment for diseases[9] and many efforts have been made to discover new antimicrobial compound from various kind of plants[10]. Chemical studies of medicinal plant provide valuable material with enormous therapeutic potential and heal many infectious diseases[6,11]. According to the World Health Organization, 80% of people in developing countries still depend on local medicinal plants to fulfill their primary health needs[12]. Besides that, there is a global consensus on the benefits of phytopharmacy and at present, medicinal plants occupy a key position in plant research and medicine.

Since anti-biotic resistance has been increasing in prevalence and becoming a major medical challenge worldwide and posing a big threat to human society, ranging from rise in the number of anti-biotic resistant bacteria to emergence of new types of resistance (particularly in Gram-negative bacteria, e.g., *E. coli* and *Klebsiella pneumoniae*), high cost of treating infectious diseases and market failure in antibiotic development, there is need to necessitate a search for novel antibacterial agents from plants extracts which are abundant, accessible and cheaper especially in the rural areas that might resolve the antibiotic resistance.

MATERIALS AND METHODS

Plant sampling and authentication: The plant *Mitracarpus villosus* was obtained from Aliero town, Kebbi state, Nigeria in July, 2018. The plant sample was identified and authenticated in the Botany unit, Biological Sciences Department, Kebbi State University of Science and Technology, Aliero, Nigeria and was found to belong to the family Rubiaceae with a voucher number 139.

Multi-drug-resistant clinically isolated bacteria: The multidrug-resistant bacteria (*Staphylococcus aureus*, *Klebsilla pneumoniae*, *Streptococcus pneumoniae and Salmonella typhi*) were obtained on 14th August, 2018 from Sir Yahaya Memorial Hospital, Birnin Kebbi, Nigeria. Sensitivity tests were conducted on the test organisms, to confirm their multi-drug-resistance.

Preparation of plant sample: The whole plant sample was cleaned and air dried at room temperature for 7 days. The completely dried sample was crushed to coarse powder by grinding with wooden mortar and pestle. The ground sample was use for the preparation of acetone extract.

Preparation of extract: The acetone extract of the plant was obtained according to the method described by Adamu *et al.*[13] with slight modification. A 200 g of the dried powder of the plant sample was dissolved in 500 mL acetone. The mixture was gently stirred, tightly covered with cotton wool and aluminium foil and was allowed to stand for 72 h at room temperature. The extract was decanted and filtered through muslin cloth. The filtrate obtained was allowed to evaporate at room temperature with continuous weighing until a constant weight was obtained. The plates were incubated in an incubator at 37°C for 24 h to get sub-cultures of the isolates.

Preparation of inoculums: After the sub-culturing, to prepare the bacterial inoculums, the sub-cultures were then inoculated on fresh nutrient agar plates using sterile cotton

swabs at 37°C for 24 h. The pure cultures on the nutrient agar plates were used as the inoculums.

Qualitative phytochemical screening: About 5 g of the plant extract was dissolved in 40 mL of distilled water and then subjected to phytochemical screening using the method of Mbatchou and Kosoono[14]. The presence of flavonoids, phenols, tannins, saponins, alkaloids, terpenoids, steroids and anthraquinones were tested.

Determination of anti-bacterial activity: The Agar well diffusion method of Pelczar *et al.*[15] was used. The Mueller-Hinton agar media were prepared according to the method described by Joklik and Willett[16]. The prepared agar plates were inoculated with the test organisms. Four wells (holes) were made into the set agar in Petri-dishes containing the inoculums using a sterile syringe of 10 mm diameter. The different concentration (75, 100 and 120 mg mL^{-1}) of the extract were prepared. A 0.25 mL (5 drops) volume of each prepared concentrations of the extract was dispensed into the different agar wells in the media followed by incubation at 37°C for 24 h. Cloxacin was used as the positive control while the solvent (acetone) was used as negative control.

Measuring zones of inhibition: After the incubation period, the plates were observed for zones of inhibition (indicated by clear zones) around the wells. The anti-bacterial activities of the extract were accessed by measuring the diameter of the zone of inhibition in millimeter around the wells using a transparent measuring ruler. The actual zone of inhibition was calculated by subtracting the diameter of the well from the measured diameter.

Determination of minimum inhibitory concentration (MIC): The minimum inhibitory concentrations of the plant extract were determined, according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. About 6 test tubes were labelled 1-6, to test tubes 2-6 1 mL of sterile nutrient broth were added, 1 mL of the extract were added to test tube 1 and test tube 2. Doubling dilution was done from tube 2-5 to have extract concentrations (in mg mL^{-1}) of 120, 60, 30, 15 and 7.5, respectively. Test tube 6 contains 1 mL of sterilized nutrient broth (serving as control for the sterility of the medium). Afterwards, 0.1 mL of a 0.5 McFarland standard of the test organisms in normal saline (0.85% NaCl (w/v)) were inoculated into the test tubes 1-5, shaken and incubated at 37°C for 24 h. Minimum inhibitory concentration is defined as the lowest concentration (in μg mL^{-1}) of an anti-biotic that inhibits the growth of a given strain of bacteria[17].

Data analysis: The data collected for the anti-bacterial activities were subjected to one way analysis of variance (ANOVA) and statistical difference between the means were separated using New Duncan's Multiple Range Test at $p<0.05$ with the aid of a statistical package (IBM SPSS Statistics 20).

RESULTS

The phytochemical composition of the *Mitracarpus villosus* extract shows that saponins, tannins, alkaloids, terpenoids, steroids, anthraquinones, flavonoids and phenols were present (Table 1).

Table 2 presents the anti-biotic sensitivity of the test organisms so as to confirm their multidrug resistance. The results indicated that cloxacin was effective against all the test organisms (*K. pneumonia, S. aureus, S. pneumoniae* and *Salmonella typhi*).

The result for the anti-bacterial activity of *M. villosus* extract (Table 3) reveals that, activity against all the test micro-organisms was exhibited at each extract concentration. It was observed that the higher the concentration of the plant extract the higher the activity. Thus, at lower concentration of the extract, activity of *M. villosus* reduces significantly. The extract exhibited highest growth inhibition on *K. pneumoniae* (6.89±0.70) and lowest on *S. pneumoniae* (5.22±0.19) at 120 mg mL^{-1}. Compared to the control drug (Cloxacin), the activity of the plant extract is significantly very low even at the highest concentration of 120 mg mL^{-1}.

Table 4 presents the minimum concentration of the extract at which anti-bacterial activity is observed. The concentration of 15 mg mL^{-1} is the minimum at which the growth of *Klebsiella pneumoniae* and *Salmonella typi* was inhibited. But, 30 mg mL^{-1} was the minimum extract

Table 1: Phytochemical composition of *Mitracarpus villosus* extract

Phytochemicals	Observation
Flavonoids	+
Alkaloids	+
Saponins	+
Tannins	+
Terpenoids	+
Steroids	+
Phenols	+
Anthraquinones	+

+: Present

Table 2: Sensitivity test results of the test organisms

	Antibiotic (50 mg mL^{-1})								
Test organisms	A	P	CH	CL	E	S	G	T	AM
Klebsiella pneumonia	-	-	-	+	+	-	-	+	-
Staphlococcus aureus	-	-	+	+	-	+	+	+	+
Streptococcus pneumonia	-	-	-	+	+	-	-	-	-
Salmonella typhi	-	-	+	+	-	+	+	+	-

+: Effective, -: Resistant, A: Augmentin, P: Penicillin, CH: Chloramphenicol, CL: Cloxacin, E: Erythromycin, S: Sivooflaxacin, G: Gentamicin, T: Tetracycline, AM: Amoxicillin

Table 3: Anti-bacterial activities of *Mitracarpus villosus* plant extract

	Zone of inhibition (mm)			
Test organisms	Cloxacin (75 mg mL^{-1})	Extract (75 mg mL^{-1})	Extract (100 mg mL^{-1})	Extract (120 mg mL^{-1})
Staphylococcus aureus	21.11±0.70^{d}	3.89±0.70^{a}	5.20±0.55^{b}	5.89±0.38^{c}
Streptococcus pneumoniae	23.00±1.76^{d}	2.44±0.84^{a}	4.00±0.58^{b}	5.22±0.19^{bc}
Klebsiella pneumoniae	20.44±5.87^{d}	4.11±1.26^{a}	5.67±1.53^{b}	6.89±0.70^{c}
Salmonella typhi	22.55±2.91^{d}	3.11±0.84^{a}	5.22±0.51^{b}	6.11±0.70^{c}

Values are presented as Mean±Standard deviation of triplicates. Values carrying different superscripts from the control (Cloxacin) for each row are significantly different ($p<0.05$) using ANOVA and Duncan multiple range test

Table 4: Minimum inhibitory concentration (MIC) of *Mitracarpu svillosus* plant extract

Test organisms	MIC (mg mL^{-1})
Staphylococcus aureus	30
Streptolococcus pneumoniae	30
Klebsiella pneumoniae	15
Salmonella typhi	15

concentration for the inhibition of the growth of *Staphylococcus aureus* and *Streptolococcus pneumoniae*.

DISCUSSION

The phytochemical composition of the *M. villosus* acetone extract indicated the presence of terpenoids, flavonoids, tannins, saponins, alkaloids and phenols. These metabolites are reported to have anti-bacterial activities in their pure form and are responsible for the anti-bacterial activities of many plants extracts[18,19]. In a research reported by Sani *et al.*[20], hexane extract of *M. scaber*, contains all these tested metabolites, except alkaloid. In comparing, the contrariety of this phytochemical composition in terms of alkaloid content might be due to the differences in species of the plant, environmental conditions and geographical locations of the places where the plant materials were obtained, the use of different solvents, procedure in the extraction or method adapted for detection of these metabolites[20,21].

The anti-bacterial activity of plant extracts earlier linked to the presence of tannins, alkaloids, flavonoids or saponins[20], tannins have been reported to inhibit growth of micro-organisms by precipitating microbial pattern and making nutritional proteins unavailable for them[22]. Similarly, the possible mechanism of action of tannins have been linked to interference with energy generation by uncoupling oxidative phosphorylation or interference with glycoprotein of cell surface[22,23]. The results indicated that, *M. villosus* has good antibacterial activity against the tested resistant bacterial isolates. Hence, the anti-bacterial activity of the plant extract might be attributed to the presence of these active metabolites[24]. This result is also in accordance with the result previously reported by Oghenejobo *et al.*[25] and Sani[19], that the plant extract has antibacterial activity on these tested organisms.

Plant species used as medicines have received a great attention in recent times due to the side effects and the serious issues of resistance that pathogenic micro-organisms develop against conventional anti-biotics. Anti-microbials of plant source are also quite active in the treatment of infectious diseases while simultaneously easing the numerous side effects often associated with synthetic anti-microbials[18]. The primary benefit of using plant medicines is that they are relatively safer and cheaper than their synthetic counterparts[26]. In addition, plant medicine is a complex combination of different phytochemicals acting by different mechanisms, which makes it difficult for pathogens to develop resistance[6].

According to WHO[12], medicinal plants would be the best source for obtaining variety of drugs. For this reason, researchers are increasingly turning their attention to herbal products, looking for new leads to develop better drugs against multidrug resistant strains. A huge number of medicinal plants have been recognized as valuable resources of natural anti-bacterial compounds. Considerable numbers of studies have been conducted on the anti-bacterial activity of

medicinal plants which showed promising effectiveness against multi-drug resistant microorganisms after antibiotics failed to eliminate them[19,26].

Minimum inhibitory concentration (MIC) is defined as the lowest concentration of any substance/antibiotic (μg mL^{-1}) that inhibits the growth of a given strain of bacteria[17]. Hence, the results for the minimum inhibitory concentration of the plant extract, reveals the MIC value (mg mL^{-1}) at which the test organisms were sensitive.

CONCLUSION

This research study discovered that *Mitracarpus villosus* whole plant extract has the ability to inhibit the growth of the multidrug-resistant clinically isolated bacterial strains (*Staphylococcus aureus*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae* and *Salmonella typhi*). Furthermore, the plant has very promising anti-bacterial activity and thus can be beneficial for the treatment of various infectious diseases cause by these resistant bacteria. This finding can serve as lead to researchers for the development of effective, safe, readily available and affordable antibacterial drug.

SIGNIFICANCE STATEMENT

This research study discovered that *Mitracapus villosus* can be used for the treatment of bacterial infectious diseases especially those cause by the tested strains. As the current situation of antibiotic resistance is dingy and resistance mechanisms are pandemic, this creates a huge clinical and financial burden on the healthcare system worldwide. If it continues, it may result in pathogens that evade all existing therapeutic agents. The prevalent dissemination of antibiotic resistance genes should provide sufficient caution for implementation of new antibiotic alternatives more especially from plant resources. Hence, this research can be beneficial as a guide in the search for new antibacterial agents. Novel mechanisms and innovative bold solutions must be obtained by researchers in order to slow down the rate of resistance. If not, the pre-antibiotic era awaits next generation.

REFERENCES

1. Fu, W., K. Lee and S. Wang, 2010. Antibiotic Resistance. In: Herbs and Natural Supplements: An Evidence-Based Guide, Braun, L. and M. Cohen (Eds.). 3rd Edn., Vol. 2, Elsevier, Australia, ISBN-13: 9780729539104, pp: 989-994.
2. Okeke, I.N., R. Laxmaninarayan, Z.A. Bhutta, A.G. Duse and P. Jenkins *et al*., 2005. Antimicrobial resistance in developing countries. Part I: Recent trends and current status. Lancet Infect. Dis., 5: 481-493.
3. Bandow, J.E., H. Brotz, L.I.O. Leichert, H. Labischinski and M. Hecker, 2003. Proteomic approach to understanding antibiotic action. Antimicro. Agents Chem., 47: 948-955.
4. Masoud, E.A. and H.A. Gouda, 2013. Effect of some natural plant extracts against gram negative bacteria in Njran Area, Saudi Arabia. Egypt. Acad. J. Biol. Sci., 4: 85-92.
5. Davies, J and D. Davies, 2010. Origins and evolution of antibiotic resistance. Microbiol. Mol. Biol. Rev., 74: 417-433.
6. Sani, I., R.A. Umar and S.W. Hassan, 2018. Potential alternatives for resolving bacterial antibiotic resistance. Eur. J. Pharm. Med. Res., 5: 25-34.
7. Bryskier, A., 2005. Antimicrobial Agents: Antibacterials and Antifungals. 2nd Edn., ASM Press, Washington, DC., USA., ISBN-13: 9781555812379, Pages: 1426.
8. Brotz-Oesterhelt, H. and N.A. Brunner, 2008. How many modes of action should an antibiotic have? Curr. Opin. Pharmacol., 8: 564-573.
9. Rangan, C. and D.G. Barceloux, 2009. Food additives and sensitivities. Disease-a-Month, 55: 292-311.
10. Manisha, T. and K. Vibsha, 2004. Medicinal Plants. Tandon Publishers, India.
11. Abdulhamid, A., I.M. Fakai, I. Sani, A.U. Argungu and F. Bello, 2014. Preliminary phytochemical and antibacterial activity of ethanolic and aqueous stem bark extracts of *Psidium guajava*. Am. J. Drug Discov. Dev., 4: 85-89.
12. WHO., 2002. Innovative care for chronic conditions: Building blocks for action. Global Report, Noncommunicable Diseases and Mental Health, World Health Organization Geneva, Switzerland.
13. Adamu, L.G.O., B. Edeghagba, O.M. Abiola, A.I. Elijah and O.T. Ezeokoli, 2013. Antimicrobial activity of extracts of *Jatropha curcas* and *Calotropis procera* leaves against pathogenic isolates from motorcycle helmets in Lagos metropolis. Int. J. Curr. Microbiol. Applied Sci., 2: 292-302.
14. Mbatchou, V.C. and I. Kosoono, 2012. Aphrodisiac activity of oils from *Anacardium occidentale* L. seeds and seed shells. Phytopharmacology, 2: 81-91.
15. Pelczar, M.J., E.C.S. Chan and N.R. Krieg, 1993. Microbiology: Concepts and Applications. McGraw Publication, New York, USA., ISBN-13: 9780070492585, pp: 180.
16. Joklik, W.K. and H.P. Willett, 1976. Zinsser Microbiology. 16th Edn., Appleton Century Crofts, New York, USA., ISBN-13: 9780139839733, pp: 57.
17. Witte, W., 2004. International dissemination of antibiotic resistant strains of bacterial pathogens. Infect. Genet. Evol., 4: 187-191.
18. Iwu, M.M., A.R. Duncan and C.O. Okunji, 1999. New Antimicrobials of Plant Origin. In: Perspectives on New Crops and New Uses, Janick, J. (Ed.). ASHS Press, Alexandria, VA., USA., ISBN-13: 9780961502706, pp: 457-462.

19. Sani, I., 2014. Application of medicinal plants to overcome antibiotic resistance in some selected multi-drug resistant clinical isolates. Res. Rev.: J. Pharm. Phytochem., 2: 48-52.
20. Sani, I., F. Bello and D. Abdul-Kadir, 2014. Phytochemical screening and antibacterial activity of *Allium sativum*, *Calotropis procera*, *Acacia nilotica* and *Mitracarpus scaber* mixed hexane extracts. World J. Pharmaceut. Res., 3: 142-149.
21. Levine, J.D., N.C. Gordon, J.C. Bornstein and H.L. Fields, 1979. Role of pain in placebo analgesia. Proc. Natl. Acad. Sci. USA., 76: 3528-3531.
22. Abdulhamid, A. and I. Sani, 2016. Preliminary phytochemical screening and antimicrobial activity of aqueous and methanolic leave extracts of *Balanites aegyptiaca* (L.). Int. Res. J. Pharmaceut. Biosci., 3: 1-7.
23. Roy, H., A. Chakraborty, S. Bhanja, B.S. Nayak, S.R. Mishra and P. Ellaiah, 2010. Preliminary phytochemical investigation and anthelmintic activity of *Acanthospermum hispidum* DC. J. Pharmaceut. Sci. Technol., 2: 217-221.
24. Sani, I. and A. Abdulhamid, 2016. Phytochemical compositions and antibacterial activities of some medicinal plants found in Aliero Area, Kebbi State, Nigeria. Int. J. Pharma Sci. Res., 7: 82-87.
25. Oghenejobo, M., K.E. Uvieghara and E. Omughele, 2013. Antimicrobial activities and phytochemical screening of the leaf extract of *Mitracarpus scaber*. Scholars Acad. J. Biosci., 1: 227-230.
26. Abdulhamid, A., I.M. Fakai, I. Sani, F. Bello and A.A. Hassana, 2016. Phytochemical screening and antibacterial activity of aqueous leaves and stem bark extracts of *Vernonia amygdalina*. Acta Velit, 3: 68-72.