2018| Volume 5 | Issue 1 |p. 74 – 78

Ewemen Journal of Microbial Research

ISSN: 2488-9148

Available online at http://ewemen.com/category/ejmr/

Original Research

PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL ACTIVITY OF METHANOL EXTRACT OF THE STEM OF *PENNISETUM GLAUCUM* LINN

[1]SODIPO O.A., [2]ABBAS T.D., [3*]YAKUBU J., [3]GULANI I.A.

[1]Department of Clinical Pharmacology and Therapeutics, College of Medical Sciences, University of Maiduguri, PMB 1069, Maiduguri, Borno State, Nigeria.
[2]Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Maiduguri, PMB 1069, Maiduguri, Borno State, Nigeria.
[3]Department of Chemistry, Faculty of Science, University of Maiduguri, PMB 1069, Maiduguri, Borno State, Nigeria.

Received 25 Mar, 2019
Revised 12 Apr, 2019
Accepted 29 Apr, 2019

**Corresponding Author's Email: jamesyakubu96@gmail.com*

ABSTRACT

The study is aimed at testing the antimicrobial activity of methanol stem extract of *Pennisetum glaucum*. Cold extraction was used to extract the powdered stem of the plant using methanol. The extract was dried and the percentage yield obtained was 4.3% (w/w). The result obtained showed the extract did not exhibit any activity against Gram positive bacteria (*Staphylococcus aureus and Streptococcus pyogenes*), Gram negative organisms (*Escherichia coli and Salmonella typhi*) and fungi (*Candida albicans and Aspergillus niger*) tested. The results of the phytochemical analysis revealed the presence of carbohydrates, tannins, cardiac glycosides, flavonoids and alkaloids in the methanol extract of *P. glaucum* stem. The result of antimicrobial study did not conform to earlier reports by other researchers. The fact that the extract of the plant did not show any antimicrobial activity on microbes used for this study may be due to growing challenge of antimicrobial resistance and the species variation due to differences in geographical location of the plant.

Keywords: *Pennisetum glaucum* Linn; Stem; Phytochemicals; Antibacterial.

INTRODUCTION

Antibacterials are used to treat bacterial infections. The drug toxicity to humans and other animals from antibacterials is generally considered low. Prolonged use of certain antibacterials can decrease the number of gut flora, which may have a negative impact on health. Consumption of probiotics and reasonable eating can help to replace destroyed gut flora. Stool transplants may be considered for patients who are having difficulty recovering from prolonged antibiotic treatment, such as recurrent *Clostridium difficile* infections (Brandt, 2013).

The discovery, development and use of antibacterials during the 20th century have reduced mortality from bacterial infections. The antibiotic era began with the pneumatic application of nitroglycerine drugs, followed by a "golden" period of discovery from about 1945 to 1970, when a number of structurally diverse and highly effective agents were discovered and developed. Since 1980 the introduction of new antimicrobial agents for clinical use has declined, in part because of the enormous expense of developing and testing new drugs (Ventola, 2015).

Foods which contain phytochemicals have been found to provide protection against diseases such as hypertension, diabetes, cancer and heart diseases. They have also been found to have oxidative properties (Adom and Liu, 2002; Grimmer *et al.,* 1992). According to Daniel *et al.* (2011), phytochemicals in millets are considered to be anti-nutritional and therefore neglected; however there is renewed interest in these compounds because of their already known health benefits. Pearl millet is the hardiest warm season grain which can survive in adversely poor conditions. The cereal can survive in very poor soils, most dry regions, saline soils and very hot regions in the world (Amarender *et al.,* 2013).

Pennisetum glaucum (pearl millet) is one of the earliest indigenous food crops in Kenya and East Africa in general. The grain is rich in proteins, lipids and micronutrients. It is a potential source of energy containing 361 kcal/100 g which is slightly higher when compared to other indigenous cereals and more than two times that is contained in maize. The content of micronutrients in pearl millet is also higher than that of maize and rice (Reddy *et al.*, 2013). Most of millet genera are widely distributed throughout the tropics and subtropics of the world (De Wet, 2006). The plant can survive in arid regions with soils of low fertility (Girish *et al.,* 2015). Pearl millet has been used as a food crop for thousands of years in a variety of food products, and continues to be used as a staple grain by approximately 90 million people in Africa and India (Gulia *et al*., 2007). It contains more nutrients than rice or wheat, but is considered a subsistence crop for poorer countries (Kajuna, 2001). Pearl millet grain contains higher gross energy than corn, higher concentrations of amino acids, and 27–32% more protein (Gulia *et al.,* 2007). As a gluten-free food product, pearl millet is becoming popular in the growing healthy food market (Gulia *et al.,* 2007).

The World Health Organization (WHO) in its efforts to promote indigenous knowledge has recommended standardization of traditionally used foods and medicinal plants as a way of improving quality. Determination of the active compounds in plants used traditionally in treatment of diseases and/or nutraceuticals is one of the standardization elements. This creates the need to isolate and determine the structure of the bioactive compounds in Pearl millet. These will act as "markers" for quality, therefore, increasing their acceptability back to the communities which initially used them and beyond (Ndiku and Ngule 2016).

According to Amarender *et al* (2013) the share of pearl millet used as cattle feed, poultry feed, alcohol industry and other non-food uses is likely to increase while the use of the grain as part of human diet is likely to decrease by 2020. This being equally dangerous in light of the great nutritional and health benefits which can be derived from pearl millet as compared to other cereals such as maize, will most likely lead to reduction in the nutritional and health benefits derived from pearl millet. Hence, the comparative study on the antibacterial and phytochemical composition of pearl millet and maize.

In developing countries especially in Nigeria, infectious disease is one of the causes of death, especially in children. As such, there is a need to explore and investigate the medicinal plant for their antimicrobial activity. *Pennisetum glaucum* is abundant especially in Maiduguri. The main aim of the study was to determine the antimicrobial activity of the methanol extract of *P. glaucum* against some Gram-positive and Gram-negative organisms. However, the objectives of the study were to determine the phytochemical constituents of the methanol extract of *P. glaucum,* evaluate the antimicrobial activity of the plant extract by determining zones of inhibition and determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against the test organisms.

MATERIALS AND METHODS

Materials

All reagents were of analar grade and products of JHD chemical product (India). The culture media used in this study was nutrient agar (Biotec Medical Market, UK) for the bacteria and peptone water (Bangalor Fine Chemicals, India). The organisms were clinical isolates obtained from the Department of Microbiology and Parasitology, Faculty of Veterinary Medicine, University of Maiduguri, Nigeria, the antibiotic drugs; Ciprofloxacine and Tetracycline (Novartis, Canada).

Plant collection and identification

Fresh Stem of *P. glaucum* was obtained from Dalori village of Konduga Local Government Area, Borno state, Nigeria. The plant was identified and authenticated by a Plant Taxonomist, Prof. S. S. Sanusi, of the Department of Biological Sciences, University of Maiduguri.

Preparation of plant extract

The fresh stem of *P. glaucum* was cleansed and air-dried under shade for seven days. It was then pulverized into powder using mechanical grinding machine. The powdered material was stored in an airtight container prior to the extraction process. Methanol was used as the solvent of extraction.

Extraction

The method used for the extraction process is maceration. Five hundred grams (500 g) of the powdered plant material was weighed and transferred into an amber coloured bottle. A sufficient quantity of methanol was poured onto the powder and soaked for three days. After every 24hrs, the bottle was vigorously shaken to facilitate the extraction. The extracted sample was filtered and allowed to dry on a stainless steel tray. The extract was then scrapped off, weighed and stored.

Phytochemical Screening

A small quantity of the extract was subjected to phytochemical screening to detect the presence of carbohydrates, tannins, cardiac glycosides, saponins, alkaloids and flavonoids using procedures described by Evans, (2009).

Antimicrobial studies

Test organisms

A total of six microorganisms were used in this study; two of which are Gram positive organisms (*Staphylococcus aureus and Streptococcus pyogenes*), two Gram negative organisms (*Escherichia coli and Salmonella typhi*) and two fungi (*Candida albicans and Aspergillus niger*). The organisms were clinical isolates obtained from the Department of Microbiology and Parasitology, Faculty of Veterinary Medicine, University of Maiduguri, Nigeria.

Sterilization of material

The media was sterilized in an autoclave at 121°C for 15 minutes. Pipettes and other glass wares were sterilized by dry heat in hot air oven at 160°C for one hour. The surface of the working area was disinfected prior to the procedure. The disc used for the standard and extract preparation (punched Whatman No. 1filter paper) was sterilized in a hot air oven at 60 °C for 30 minutes.

Antimicrobial susceptibility test

The antimicrobial susceptibility test was carried out using a concentration of 100, 200, 300, 400 and 500 mg/ml. The clinical isolates of the microorganism were diluted in normal saline. About 0.5 ml of each of the dilute cultures was aseptically inoculated on the surface the sterile petri dish containing sterile solidified nutrient agar. Discs impregnated with the plant extract were aseptically mounted on the inoculated agar and incubated at 37°C for 24 hours. The zones of inhibition was observed and then recorded in millimetres using a transparent ruler and the results were recorded. The standard antimicrobial drug used were Ciprofloxacin (5 µg) and Tetracycline (30 µg) (Sodipo, 2012).

RESULT AND DISCUSSION

Extraction yield

The weight, colour, texture, and percentage yield of the extracts obtained from reflux extraction are presented in Table 1. The colour of the methanol extract was dark brown, the texture was oily, has aromatic odour, the weight of the extract was 21.5 g, and the percentage yield was 4.3 % ($^{w}/_{w}$).

Table 1: Physical characteristics and percentage yield of methanol extract of *Pennisetum glaucum* stem

Physical parameters	Result
Colour	Dark brown
Odour	Aromatic
Texture	Coarse
% yield	4.3

Phytochemical Constituents of Stem of *P. glaucum*

The result of the phytochemical screening of the methanol extract of *P. glaucum* stem is shown in Table 2. The result showed the presence of carbohydrates, tannins, cardiac glycosides, flavonoids and alkaloids. However, saponins, terpenoids and anthraquinones were absent.

The presence of these phytochemicals is in accordance with previous studies which reported the presence of these phytochemicals in the same plant (Abdurohaman, 2015). The presence of carbohydrate, as a primary metabolite, in the present study agrees with the previous work that has reported carbohydrates as phytochemicals of plant (Sukhvinder, 2014). The presence of cardiac glycoside, in the present studies is in agreement with the previous work carried out to

detect the presence of tannins, cardiac glycosides, flavonoids and alkaloids (Sukhvinder, 2014).

Table 2: Phytochemical Screening of the Methanol Extract of *P. glaucum*

Class of metabolite	Phytochemical Test	Observation	Inference
Carbohydrates	Molisch test (General test)	Purple colour	+
	Test for reducing sugar-Fehling's test	Brick-red precipitate	+
	Test for combined reducing sugar	Reddish brown precipitate	+
	Test for ketoses	No colour change	-
Tannins	Ferric chloride test	No colour change	-
	HCl	Red precipitate	+
Phlobatannins	HCl test	No form formation	+
Cardiac glycosides	Salkwoski test	Reddish brown precipitate	+
	Liebermann-Burchard's test	No colour change	-
Flavonoids	Shinoda's test	No change in colour	-
	Ferric chloride test	Green-blue colour	+
	Sodium hydroxide	Colourless	+
Saponins	Frothing test	No form formation	-
Alkaloids	Dragendroff's reagent	Orange red precipitate	+
	Meyer's reagent	Buff-red coloured precipitate	+
Anthraquinones	Borntrager's test for free Anthraquinones	Red colour	+
	Test for combined anthraquinones	Violet colour	+
Terpenoids	Acetic anhydride test	Pink colour	+

Key: - = Absent; + = Present

Antimicrobial Sensitivity Test of the Methanol Extract of *P. glaucum*

The result of the *in vitro* antibacterial sensitivity test of the extract on the selected microorganisms is shown in Table 3. The result of the *in vitro* disc diffusion antibacterial activity showed no zones of inhibition against Gram positive bacteria (*S. aureus* and *S. pyrofen*), Gram negative bacteria (*E. coli* and *S. typhi*) and fungi (*C. albian* and *A. niger*) at concentrations of 100, 200, 300, 400 and 500 mg/ml. This result contradicts the work reported by Ndiku *et al.* (2016). The disparity in the result of this work with that of the study reported by Ndiku *et al* (2016), Amadou *et al* (2011) and Brandt (2013), may be due to antimicrobial resistance and difference in the chemical profiles due to difference in geographical location of plant collected and used in this work.

According to study reported by Abdel-Karim *et al* (2017), *P. glaucum* oil showed excellent activity against isolated *Bacillus subtilis*, *Staphylococcus aureus*. It also has good activity against the fungal species *Candida albicans* and moderate against *Aspergillus niger*. Antimicrobial properties of the seed extract studied by Singh *et al* (2015) using Kirby Bauer well diffusion technique showed that polyphenols of millet had antimicrobial activity on *Escherichia coli, Staphylococcus aureus, Proteus mirabilis, Pseudomonas aeruginosa, Serratia marcescens, Klebsiella pneumoniae, Shigella dysenteriae, Enterococcus sp. and Salmonella* sp.

Table 3: *In vitro* Antimicrobial Activity of the Stem Extract of *Pennisetum glaucum*

Organisms	Concentration of Extract (mg/ml)					Standard Antibiotic discs	
	100	200	300	400	500	TCN (30µg)	CIPRO (5µg)
S. aureus	-	-	-	-	-	-	27.3
S. pyrofen	-	-	-	-	-	-	28.3
E. coli	-	-	-	-	-	-	28.3
S. typhi	-	-	-	-	-	-	18.0
C. albicans	-	-	-	-	-	-	19.6
A. niger	-	-	-	-	-	-	-

Key: - = No Activity; CIPRO = Ciprofloxacin; TCN = Tetracycline; Results of the control is expressed as Mean

CONCLUSION

The result of antimicrobial sensitivity of the stem extract of *P. glaucun* against selected microorganisms showed no zones of inhibition indicating no activity against the test microorganisms. However, the results of the phytochemical analysis revealed the presence of carbohydrates, tannins, cardiac glycosides, flavonoids and alkaloids in the methanol extract of the stem. Given the presence of these important chemical constituents in the methanolic extract of *P. glaucum* stem, further research should be geared towards the reason for the difference in the result obtained compared to earlier reports from studies by other researchers. Also, quantitative phytochemical studies should be carried out to ascertain the concentration of the phytochemicals present in the plant and to know the

reason of the inactivity of the methanol extract used in this study.

ACKNOWLEDGEMENT

The authors appreciate the effort and assistance of Mr. Fine Akawo of the Department of Chemistry, University of Maiduguri, Maiduguri, Borno State for the phytochemical analyses.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Abdurohaman M (2015). Phytochemical extraction and screening of bioactive compounds from *Pennisetum glaucum* stem bark extract. *Am J Life Sci,* **3**(5): 358-364.
2. Adom KK and Liu RH (2002). Antioxidant activity of grains. *Agric Food Chem*, 50(21): 6182-6187.
3. Abdel Karim M, Asma MH and Inas O (2017). GC-MS analysis and antimicrobial activity of Sudanese *Pennisetum glaucum* fixed oil. *Inter J Adv Res*, 5(4): 964-968.
4. Amarender RA, Yadav OP, Malik PD, Singh IP, Ardeshna NJ, Kundu KK, Gupta SK, Sharma R, Sawargaonkar G, Shyam DM, and Reddy SK (2013). Utilization pattern, demand and supply of pearl millet grain and fodder in Western India. International Crop Research Institute for Semi-Arid Tropics (ICRISAT). Working paper series number 37, 28p. Available at:http://hope.icrisat.org/wp-content/uploads/2015/11/Utliz-demand-and-supply-of-p.-millet-in-W.-India.pdf [Accessed 24 Mar, 2019].
5. Daniel M, Denni M and Chauhan D (2011). Polyphenols, phospholipids, and fixed oil composition of pearl millet *Pennisetum glaucum* (L.) *Inter J Pharma Life Sci,* 3(11): 2098-2102.
6. De Wet JM (2006). *Eleusine coracana* (L.): Gaertn. record from probase. In: Brink M and Betay G (eds); PROTA (Plant Resources of Tropical Africa), Washington, Netherlands.
7. Evans WC (2009). *Pharmacognosy*. 16th eds, Sanders publishers. London. pp. 42-393.
8. Girish C, Pakeshkumar M, Mahima D, Mamta K, González R, Ballester I, López-Posadas R, Suárez MD, Zarzuelo A and Martínez-Augustin O (2015), Current science effects of flavonoids and other polyphenols on inflammation. *Crit Rev Food Sci Nutr,* 51:331–362.
9. Grimmer HR, Parbhoo V, and Macgrath RM, (1992). Antimutagenicity of polyphenol-rich fractions from Sorghum bicolar grains. *Agr Food and Chem,* 59:251-256.
10. Gulia SK, Wilson JP, Carter J and Singh BP (2007). Progress in grain pearl millet research and market development. p. 196–203. In J. Janick and A. Whipkey (ed.) Issues in New Crops and New Uses. ASHS Press, Alexandria, VA.
11. Kajuna S (2001). Millet: post-harvest operations. Sokoine Univ. of Ag., Morogoro, Tanzania. D. Mejia and B. Lewis (ed.) FAO. Available at: http://www.fao.org/inpho/inphopost-harvest-compendium/cereals-grains/en/ [Accessed 08, Aug. 2014].
12. Ndiku M H, Peter G, Mwitari PG, Irungu B and Ngule C M (2016). Antibacterial activity of *Pennisetum glaucum* crude extract and purified fraction against selected pathogenic microorganism. *Br J Pharm Res,* 14(1):1-9.
13. Reddy AA, Yadav OP, Malik DP, Singh IP, Ardeshna NJ, Kundu KK, Gupta SK, Sharma R, Sawargaonkar G, Shyam DM and Reddy KS (2013). Utilization pattern, demand and supply of pearl millet grain and fodder in Western India. Working Paper Series No. 37. Working Paper. International Crops Research Institute for the Semi-Arid Tropics, Patancheru, Andhra Pradesh, India.
14. Singh N, Meenu G, Sekhar A and Abraham J (2015). Evaluation of antimicrobial and anticancer properties of finger millet (*Eleusine coracana*) and pearl millet (*Pennisetum glaucum*) extracts. *The Pharma Innovation J*, 3(11): 82-86.
15. Sodipo OA, Abdulrahman FI, Alemika TE and Gulani IA (2012). Chemical composition and biological properties of the petroleum ether extract of *Solanum macrocarpum* L. (Local Name: Gorongo). *Br J Pharma Res,* 2(2): 108-128.
16. Sukhvinder SP (2014). Phytochemical analysis of the ethanolic extracts of different pearl millet (*Pennisetum glaucum*) varieties. *J Nat Prod Plant Resour*, 4(5):19-23.
17. Ventola CL (2015). The antibiotic resistance crisis, Part 1: Causes and threats. *Pharm Ther,* 40(4): 277–283.

Article's Citation

Sodipo OA, Abbas TD, Yakubu J and Gulani IA (2019). Phytochemical analysis and antibacterial activity of methanol extract of the stem of *Pennisetum glaucum* Linn. *Ew J Microb Res* 5(1): 74 – 78.

Author's Contribution

SOA conceived and designed the study. ATD, and GIA carried out all laboratory work. ATD interpreted data and managed the literature searches. YJ wrote the initial manuscript. YJ revised the manuscript. All authors read and approved the final manuscript.