British Journal of Pharmaceutical Research
14(1): 1-9, 2016; Article no.BJPR.28434
ISSN: 2231-2919, NLM ID: 101631759

SCIENCEDOMAIN *international*
www.sciencedomain.org

Antibacterial Activity of *Pennisetum glaucum* Crude Extracts and Pre-purified Fractions against Selected Pathogenic Microorganisms

Hellen M. Ndiku[1], Peter G. Mwitari[2], Beatrice Irungu[2] and Chrispus Mutuku Ngule[2*]

[1]Department of Nutrition and Dietetics, School of Science and Technology, University of Eastern Africa, Baraton, P.O.Box 2500-30100, Eldoret, Kenya.
[2]Centre for Traditional Medicine and Drug Research, Kenya Medical Research Institute, Kenya.

Authors' contributions

This work was carried out in collaboration between all authors. Author HMN participated in conceptualization of the idea, study design and writing of the manuscript. Author PGM managed the literature searches participated in the experimental design and manuscript writing. Author BI managed the experimental process and participated in manuscript writing and author CMN participated in experimental design, management of the experimental activities, data analysis and reporting. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJPR/2016/28434
Editor(s):
(1) Huixiao Hong, National Center for Toxicological Research, U.S. Food and Drug Administration, USA.
Reviewers:
(1) Ana Carolina Oliveira da Silva Gambôa, Universidade Federal Rural de Pernambuco, Brazil.
(2) Kathleen L. Hefferon, Cornell University, United States.
(3) Piyali Datta Chakraborty, Albert David Ltd., India.
Complete Peer review History: http://www.sciencedomain.org/review-history/17058

Original Research Article

Received 19th July 2016
Accepted 3rd October 2016
Published 29th November 2016

ABSTRACT

Background: *Penisetum glaucum* is one of the earliest indigenous food crops in East Africa. Pearl millet contains numerous phytocompounds. Isolation and characterization of secondary metabolites from pearl millet and evaluation of their bioactivity is necessary. These will act as "markers" for pharmacological quality, increasing the acceptability of pearl millet. The current study was therefore undertaken to provide thin layer chromatography (TLC) profile using different solvent systems and pre-purified fractions.
Methods: Maceration and sequential extraction methods were used. Thin layer chromatography

**Corresponding author: E-mail: chrisngule@gmail.com;*

and column chromatography techniques were used in extracts purification. The antibacterial activity of the crude extracts and pre-purified fractions was determined using well diffusion method.
Results: Pearl milletethyl acetate and petroleum ether extracts gave good activity as compared to other tested crude extracts. Petroleum ether inhibited *Serratia liquefaciens* the most with an average inhibition zone of 10.66±0.882 while ethyl acetate inhibited Bacillus cereus (12.333±0.577). Methanol extract inhibited the growth of only *E. coli*. Fraction 10 which was obtained from the petroleum ether extract inhibited the growth of all the microorganisms tested. Gentamicin which was used as the positive control had significantly higher inhibition zones against all the selected microorganisms as compared to the plant test samples ($p<0.001$). DMSO which was used as the negative control did not inhibit the growth of any of the selected microorganisms.
Conclusion: The results obtained in the current investigation of *P. glaucum* show that the cereal contains secondary metabolites with useful pharmacological activities. The antibacterial activity observed in this study could be a scientific justification of the plants potency in prevention and management of gastrointestinal infections due to its ability to inhibit the growth of *E. coli*. A lot has been done on the primary metabolites of pearl millet ascertaining its richness in minerals, vitamins and primary nutritional molecules. It is therefore necessary to give attention to the secondary metabolites concentration and their pharmacological importance. Further purification, isolation, identification and characterization of pearl millet bioactive compounds remain to be our priority in future.

Keywords: Pearl millet; microbial; indigenous; chromatography; Phytochemicals.

1. INTRODUCTION

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 as 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 [1,2]. Preliminary phytochemical analysis of pearl millet hydromethanolic extract showed the grain to contain a wide spectrum of important pharmacological compounds viz: tannins, phenols, flavonoids and steroids [3]. The phenols in pearl millet have been associated with its ability to treat celiac disease and a wide range of non-communicable diseases. The fiber content in the grain has also been attributed to its treatment against constipation [4]. Despite, the numerous nutritional and pharmacological benefits which can be obtained from pearl millet the grain is at the verge of extinction due to its replacement with nutritiously inferior cereals such as maize and rice [5].

Majority of the Kenyan population live in abject poverty and are at the risk of being malnourished despite the existence of highly nutritious indigenous grains such as pearl millet. Non communicable diseases such as cancer and diabetes are on the increase in Kenya among other developing countries due to conformation to the western foods [1,6]. This creates the need for investigation of both nutritional and pharmacological value of indigenous cereals. Research based approach to unveil the nutritional and pharmacological value of indigenous cereals is necessary. This is in an attempt to increase their re-introduction and general acceptance for inclusion in the shopping list.

Pearl millet is strongly tied to the heritage of many communities in Kenya. For example the Kamba community used its flour during the long distance trading to prepare special bread called “Kimutu”. This bread could be carried for several days without getting spoiled signifying its potency to inhibit food spoilage bacteria [7].

The World Health Organization 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 [8]. 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. Currently there are no reports on phytochemical studies that have used activity-guided fractionation to isolate bioactive

compounds with antibacterial activity from pearl millet. Reports on the isolation of bioactive compounds or nutritiously important constituents from pearl millet are limited too. The current study was therefore undertaken to provide TLC profile using different solvent systems which can aid in the isolation of bioactive compounds from different extracts of pearl millet. This is an attempt to stimulate research towards isolation and elucidation of compounds available in the grain to be used as hints in understanding the biological activities attributable to pearl millet or template for allopathic pharmaceuticals for treatment of non-communicable diseases such as cancer and diabetes [9]. Pre-purified and pure compounds have also been obtained in the current study. Antibacterial activity of the crude extracts and pre-purified fractions has been done.

1.1 Study Sites

The samples extraction, thin layer chromatography and column chromatography were performed at the Centre for Traditional Medicine and Drug Research at the Kenya Medical Research Institute (KEMRI). The antibacterial activity of the samples was done at the Department of Biological Sciences, University of Eastern Africa, Baraton.

2. MATERIALS AND METHODS

2.1 Sample Acquisition and Extraction

The samples were obtained from H.M.N Ukambani project stores. The samples were ground into fine powder using a grinding miller, weighed and stored at room temperature.

2.2 Organic Solvent Extraction

Using analytical beam balance 100 g of the sample was placed into a 250 ml conical flask, 90% methanol was added until the sample was completely submerged. The mixture was agitated for thorough mixing. The extraction process was allowed to continue for 24-48 hrs with frequent shaking for effective extraction of the plant components (maceration). The mixture was vacuum filtered using Butcher funnel, whatman no. 1 filter paper with the help of a vacuum pump. The filtrate was re-filtered using the same apparatus. The solvent was then removed using rotary vacuum evaporator with a water bath at 40℃ [10]. Sequential extraction was also used. Briefly, three solvents were used viz: petroleum ether, ethyl acetate and methanol in order of their increasing polarity. The samples were first soaked in petroleum ether for 24 hours, filtered and the filtrate concentrated using rotary vacuum evaporator. This procedure was repeated for ethyl acetate and methanol respectively. The crude extract for 90% methanol was fractionated using a separator funnel in different solvents in order of their increasing polarity viz: petroleum ether, ethyl acetate and water respectively.

2.3 Thin Layer Chromatography (TLC) Analysis of the Samples

Thin layer chromatography profiling was done using TLC plates with silica gel 50 F254 TLC (Merck, Germany). A 7×6 cm was cut using a sharp pair of scissors and plate markings done using a pencil. The base line was set 1cm from the edge of the plate. A straight line was drawn to mark the base line. Different solvent systems were used to separate the compounds in the TLC paper. Different sample spots were made on the base line and the plate inserted in the development chamber containing the solvent system of choice. The solvent systems were chosen depending on the nature of the extract. After of development, the TLC plates were sprayed with vanillin for bands visualization. The solvent system that gave the best compounds separation in the TLC plate was used in the column chromatography.

2.4 Column Chromatography

The column was packed with silica gel using petroleum ether as the eluent. Using a long Pasteur pipette, the sample was added to the column and appropriate solvent system used to elute the sample component. Ethyl acetate extract fractionation was done using petroleum ether, ethyl acetate and methanol in the ratio of 7:2:1, while petroleum ether extract was fractionated using petroleum ether and ethyl acetate (8: 0.4) solvent system.

2.5 Antibacterial Assay

2.5.1 Bacteria source and media preparation

The bacteria used were commercial pure cultures from Carolina biological supply company (USA). The colonies for use in the study were obtained from the pure cultures and then transferred into blood agar plates. The plates were then incubated at 37℃ for 24 hours. The blood agar media was prepared according to the manufacturer's instructions. The plates were

sterilized by the use of an autoclave at 121℃. Approximately 20 ml of the prepared media was poured into the sterilized plates. The surface of the media was flamed using a bunsen burner flame to remove air bubbles and sterilize the media surface. Mueller Hinton broth was prepared according to the manufacturer's instructions. About 5ml of the broth was transferred into sterile test tubes. The transfer of the media to the plates and test tubes was done under sterile biohazard wood [11].

2.5.2 Preparation of the bacterial suspension

The turbidity of each of the bacterial suspension was prepared to match to a 0.5 McFarland standard [12]. The McFarland standard was prepared by dissolving 0.5 g of $BaCl_2$ in 50 ml of water to obtain a 1% solution of Barium chloride (w/v). This was mixed with 99.5 ml of 1% sulphuric acid solution. Three – five identical colonies of each bacterium were taken from a blood agar plate (Himedia) culture using a sterile swab into Mueller Hinton broth (Himedia). The broth culture was incubated at 37℃ for 2 - 6 hours until it achieved turbidity similar to the 0.5 McFarland standard. The culture that exceeded the 0.5 McFarland standard was adjusted with the aid of a UV spectrophotometer to $0.132A^0$ at a wavelength of 600 nm in order to obtain an approximate cell density of $1x10^8$ CFU/ml.

2.5.3 Preparation of the samples concentrations and antibiotic

Extracts stock solutions were prepared by dissolving 200 mg for crude extracts, 100 mg for pre-purified fractions and 20 mg of the fractions producing a single spot in 1 ml of dimethylsulfoxide (DMSO). Gentamicin octodiscs were used as the positive control. Dimethylsulfoxide seeded Kirby-Bauer discs served as the negative control.

2.5.4 Determination of bioactivity of the samples

Mueller Hinton agar plates were prepared as by the manufacturer's instruction. Briefly 0.1 ml of each of the prepared bacterial suspension for the test was transferred to 3 plates for each organism to give a triplicate for each concentration and organism. The samples were tested for antimicrobial activity using the disc diffusion method (Kirby-Bauer method) [13]. Sterile commercial blank discs (Himedia), 6.0 mm diameter, were impregnated with the crude extracts and pre-purified fractions. Discs were allowed to dry in an oven at 37℃ prior to use. Sample-impregnated discs (20 µl) were placed on agar plates and incubated at 37℃ for 24 hours. DMSO (20 µl) was used as a negative control and gentamicin commercial discs as the positive control. Three plates were made for each bacterial organism and extract giving a triplicate reading for each microorganism and extract. Zones of inhibition were measured in millimeters with the aid of a ruler.

3. RESULTS

3.1 TLC Profile and Column Chromatography Extracts Purification and Compound Isolation

Two extracts were used viz: ethyl acetate (mid-polar) and petroleum ether (non-polar). The TLC for ethyl acetate extract was done using petroleum ether, ethyl acetate and methanol in the ratio of 7:2:1. This solvent system gave best separation of the compounds on the TLC as compared to all other solvent systems tested. Five distinct bands were observed with R_f values of 0.13, 0.34, 0.8, 0.88 and 0.96 (Fig. 1). Column chromatography extracts purification was done using the same solvent system. This gave fractions with spots of different R_f values. Fractions that were initially observed to be single spots were further analyzed using different TLC techniques viz: solvent system adjustment to increase or reduce R_f values, two dimensional TLC development and double TLC development to confirm the purity of the spots. All the fractions were packed in sterile clean vials and stored at 4℃. TLC plates were preserved for future reference by marking the position of the spots and sealing the plate using a transparent tape. The petroleum ether extract gave two distinct bands using petroleum ether and ethyl acetate (8: 0.4) solvent system, with a number of compounds dragging slightly above the baseline (Fig. 2). The R_f values of the two distinct spots were 0.56 and 0.77. The other solvent systems used did not show proper separation of the compounds on the TLC viz: petroleum ether and ethyl acetate 8:2, 6:4, 5:5, 9:1 and petroleum ether, ethyl acetate and methanol, 7:3:0.5. The solvent system of petroleum ether and ethyl acetate (8:0.4) was used in the column chromatography. However, the solvent system polarity was increased gradually based on the observations noted in the TLC separation. Several fractions were obtained and analyzed using TLC. Similar fractions were combined and

re-spotted (Figs. 3 and 4). Double and two dimensional TLC techniques were also used to confirm the purity of the single spots (Fig. 5). Pre-purified fractions were dissolved in petroleum ether, put in 1.5 ml sterile clean vails and air dried. The samples were stored at 4℃.

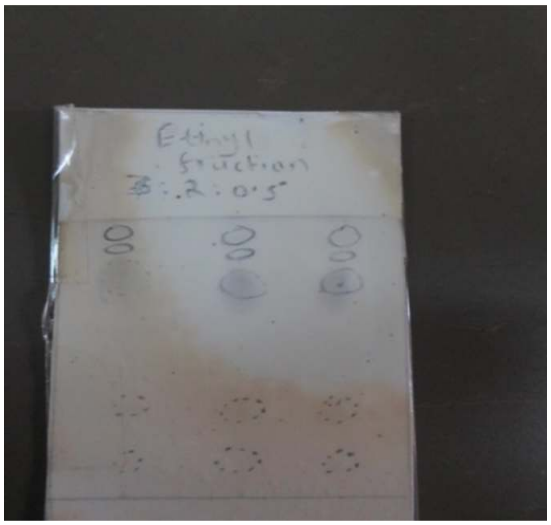

Fig. 1. Ethyl acetate extract TLC compounds separation

Fig. 2. Crude petroleum ether TLC profile using selected solvent system

The antibacterial analysis of the plant extracts and pre-purified fractions was done using disc diffusion method. The data on the zones of inhibition was obtained and analyzed using SPSS version 22. The mean zones of inhibition and standard error (S.E) were extracted from the SPSS results and presented graphically for easy visualization and data interpretation. Comparisons between the zones of inhibition caused by the samples were performed using ANOVA SPSS analysis ($p<0.05$). The degree of activity of the samples antimicrobial activity was expressed according to inhibition zone diameter as follows; resistant (<7 mm), 8–11 mm intermediate activity and >12 mm very active [14].

The plant gave impressive antibacterial activity with the ethyl acetate extract giving the best activity as compared to other tested crude extracts (Fig. 6). Methanol extract inhibited the growth of only *E. coli*. Fraction 10 which was obtained from the petroleum ether extract inhibited the growth of all the microorganisms tested. Gentamicin which was used as the positive control had significantly higher inhibition zones (Fig. 6) against all the selected microorganisms as compared to the plant test samples ($p<0.001$). Dimethyl sulfoxide which was used as the negative control did not inhibit the growth of any of the selected microorganisms. All tested extracts inhibited the growth of *E. coli* (Fig. 6).

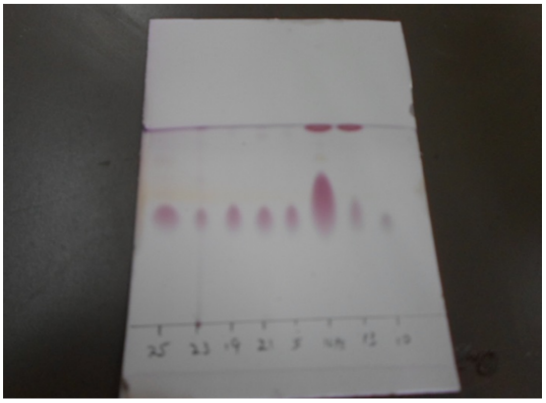

Fig. 3. TLC compounds separation after solvent system adjustment

4. DISCUSSION

Thin layer chromatography profiling of the selected pearl millet fractions gave noteworthy results indicating presence of a number of compounds in the grain. Various phytocompounds gave different retention values

(Rf) when exposed to different solvent systems. The differences in Rf values provide important information on the polarity of the compounds. This aids in separation of the compounds using column chromatography. In the current study the ethyl acetate gave five distinct bands indicating presence of five different phytochemicals. This suggests that five compounds can be isolated from the ethyl acetate extract. Adjustment of the solvent system for petroleum ether extract is essential to avoid drugging of the compounds as observed in the TLC plates. This can be achieved through analyzing of the R_f values of the present compounds in different solvent systems [15].

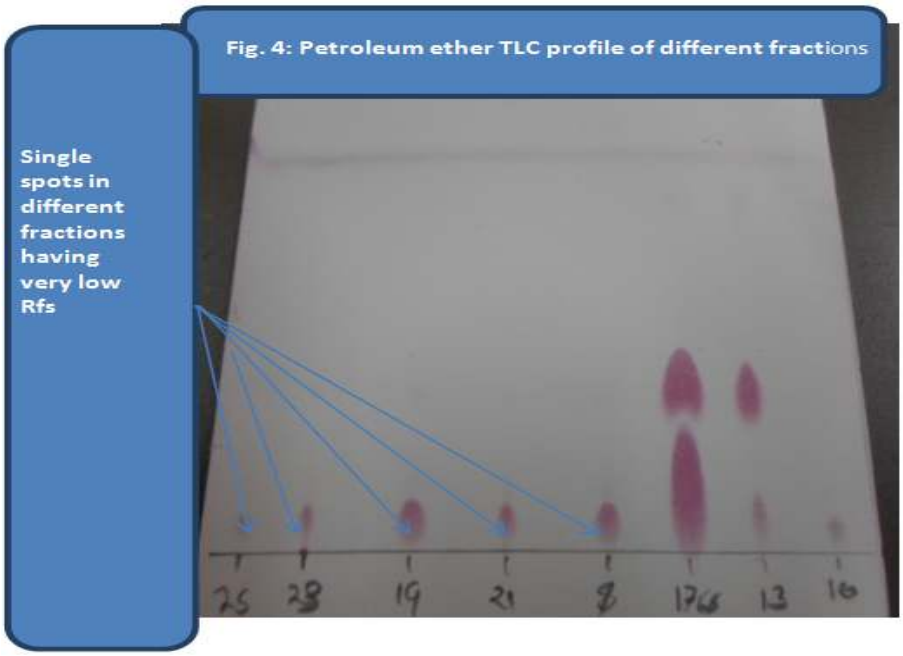

Fig. 4. Petroleum ether TLC profile of different fractions

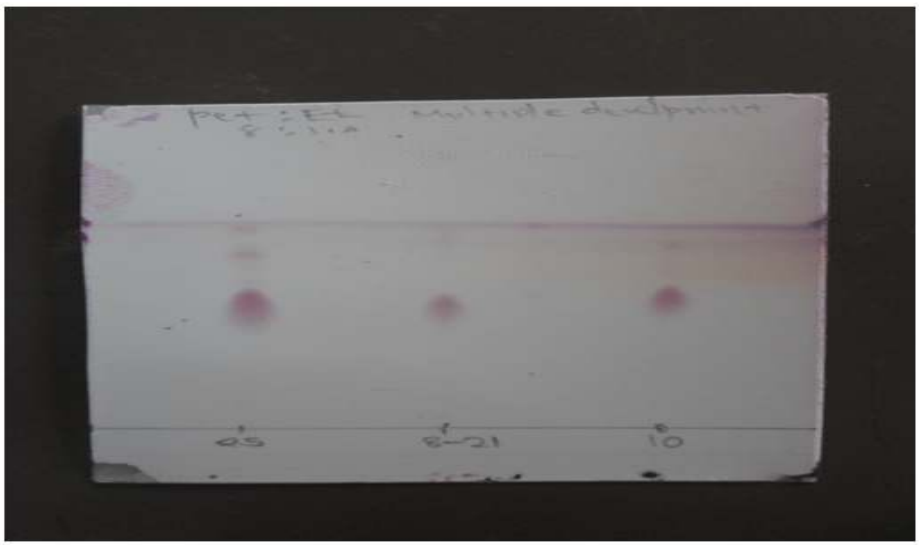

Fig. 5. Multiple TLC development for single spot confirmation

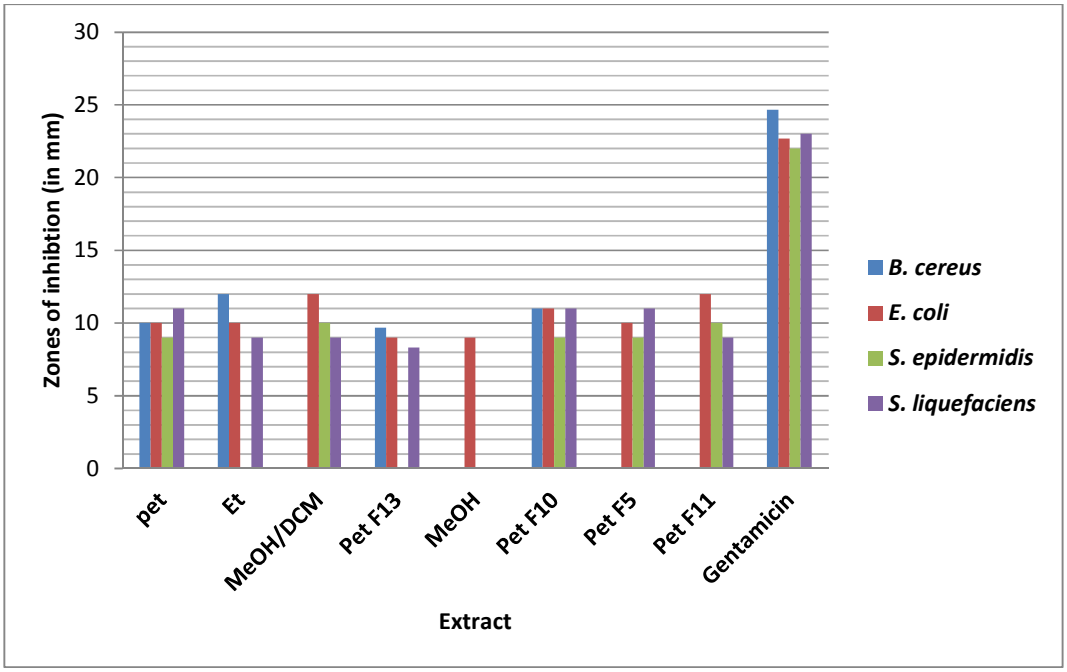

Fig. 6. Zone of inhibition of various pearl millet extracts and pre-purified fractions

The current results on the antibacterial activity of pearl millet are in conformity with previous studies. According to Singh et al. [16], finger millet hexane extract inhibited the growth of *E. coli* at 100 mg/ml concentration. The plant's ability to inhibit *E. coli* growth may be a scientific justification that the plant could be used to prevent enteric infections caused by the bacteria. The inhibition of *Staphylococcus epidermidis* by different extracts and pre-purified fractions from pearl millet is noteworthy since the bacterium has been associated with various clinical infections including oral and nosocomial infections. The intermediate inhibition of *S. epidermidis* by pearl millet may be of help in enhancing oral health. The consumption of the grain could also prevent self-limiting gastroenteritis caused by *Bacillus cereus.* The current study also conforms to our previous studies in which the plants methanolic-aqua extract did not inhibit the growth of *Serratia liquefaciens*, *E. coli* and *Bacillus cereus* as also observed in current methanol extract [3]. However, the current study is different since the methanol extract inhibited the growth of *E. coli* a difference which could be attributed to the methods used in the extraction of the phytochemicals. In the current study the methanol extract was obtained through sequential extraction. Fraction 10 showed intermediate activity against the selected bacteria. This signifies that fraction 10 which was the most common and highly concentrated compound in both TLC analysis and column chromatography obtained fractions could be used as markers for pharmacological quality of pearl millet after spectroscopy purity verification and structural elucidation.

5. CONCLUSION

The results obtained show that the cereal contains a rich source of pharmacological compounds. The antibacterial activity observed in this study could offer a scientific justification of the plants potency in prevention and management of gastrointestinal infections due to its ability to inhibit the growth of *E. coli*. This could further be justified by our previous studies in which the plants methanolic-water extract inhibited the growth of *S. typhi,* clinically important gastrointestinal bacteria [3]. The information provided in this work could help in selection of appropriate solvent systems for compounds isolation from pearl millet. This will aid in improving traditional knowledge on the quality of pearl millet hence accelerating its re-introduction and acceptance back to African arid and semi-arid regions as an important nutritive dietary food component. A lot has been done on the nutritional value of pearl millet ascertaining its richness in minerals, vitamins and primary nutritional molecules. It is therefore necessary to

give attention to the secondary metabolites concentration and pharmacological importance. Further purification, isolation, identification and characterization of pearl millet bioactive compounds remain to be our priority in future.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

DISCLAIMER

This manuscript was presented in the conference "Baraton International Interdisciplinary Research Conference"
Available:"http://ueab.ac.ke/index/downloads/birj/research_conference_2016_programme.pdf "
Date- Wednesday, 13th April 2016

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Reddy AA, Yadav OP, Malik DP, Singh IP, Ardeshna NJ, Kundu KK, Gupta SK, Sharma R, Sawargaonkar G, Shyam DM, Reddy KS. 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; 2013.
2. Daniel M, Denni M, Chauhan D. Polyphenols, phospholipids and fixed oil composition of pearl millet [*Pennisetum glaucum*] (L.). International Journal of Pharmacy & Life Science. 2011;3(11): 2098-2102.
3. Ndiku MH, Ngule CM. Comparative study on the antibacterial and chemical constituents of *Pennisetum glaucum* (pearl millet) and *Zea mays* (maize). International Journal of Nutrition and Metabolism. 2015; 7(4):46-51.
4. Vanisha S, Dhaduk JJ, Sareen N, Shahu T, Desai R. Potential functional implications of pearl millet (*Pennisetum glaucum*) in health and disease. Journal of Applied Pharmaceutical Science. 2011; 01(10):62-67.
5. Raschke V, Cheema B. Colonization, the new world order and the eradication of traditional food habits in East Africa: Historical perspective on the nutrition transition. Public Health Nutrition. 2007; 11(7):662–674.
6. Isaacson C, Mothobi P, Hale M, Tomar IK, Tyagi C, Kumar P, Choonara YE, Altini M, Pillay V. Carcinogenic nitrosamines in traditional beer as the cause of oesophageal squamous cell carcinoma in black South Africans. S.A.M.J. 2015;105: 656-658.
7. Ndiku M, Jaceldo-Seigl K, Sabate J. Dietary patterns of infant and preschool children in Mwingi and Makueni districts of Ukambani region, Eastern Kenya. African Journal of Food Agriculture Nutrition and Development. 2010;10(7): 2804-2817.
8. WHO. Quality control methods for medicinal plant material. WHO headquarters, Avenue Appia 20, 1211 Geneva 27, Switzerland; 1998.
9. Gujjeti PR, Mamidala E. Phytochemical analysis and TLC profile of *Madhuca indica* inner bark plant extract. International Journal of Scientific and Engineering Research. 2013;4(10):1507-1510.
10. Anthoney ST, Jackie O, Ngule CM. *In vitro* control of selected pathogenic organisms by Vernonia adoensis roots. International Journal of Pharmacy and Life Sciences. 2013;4(8):2855-2859.
11. Ngule CM, Ndiku HM. Phytochemical evaluation and *In vitro* antibacterial activity of methanolic-aqua extract of *Indigofera arrecta* leaves against selected pathogenic microorganisms. African Journal of Pharmacology and Therapeutics. 2014; 3(3):74-78.
12. Ngule CM, Swamy TA, Jackie KO. Pytochemical and bioactivity evaluation of senna didymobotrya fresen Irwin used by the Nandi community in Kenya. International Journal of Bioassays. 2013; 2(7):1037-1043.
13. Arullappan S, Zakaria Z, Basri FD. Preliminary screening of antibacterial activity using crude extracts of *Hibiscus rosa sinensis*. Tropical Life Sciences Research. 2009;20(2):109–118.
14. Mwitari PG, Ayeka PA, Ondicho J, Matu EN, Bii CC. Antimicrobial activity and probable mechanisms of action of

medicinal plants of Kenya: *Withania somnifera*, *Warbugia ugandensis, Prunus Africana* and *Plectrunthus barbatus.* Plos One. 2013;8(6):e65619.

15. Talukdar AD, Choudhury MD, Chakraborty M, Dutta BK. Phytochemical screening and TLC profiling of plant extracts of *Cyathea gigantean* (Wall. Ex. Hook) Haltt. and *Cyathea brunoniana*. Assam University Journal of Science and Technology. 2010; 5(1):70-74.
16. Singh N, Meenu G, Sekhar A, Abraham J. Evaluation of antimicrobial and anticancer properties of finger millet (*Eleusine coracana*) and pearl millet (*Pennisetum glaucum*) extracts. The Pharma Innovation Journal. 2015;3(11):82-86.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/17058