

Full Length Research Paper

Comparative study on the antibacterial and chemical constituents of *Pennisetum glaucum* (pearl millet) and *Zea mays* (maize)

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The use of indigenous foods dates back to the colonization of Africa. The shift from the use of indigenous foods to the use of nutritiously inferior non-indigenous cereals is a common trend in many developing countries. The adoption of global food systems has led to simplification of East African cultural practices adversely affecting peoples' health. The current study compared the antibacterial activity and phytochemical composition of *Pennisetum glaucum* (indigenous) and *Zea mays* (non-indigenous), which established that 75% out of 100% of all the phytochemicals tested were found to be present in the pearl millet grain as compared to maize, which was found to contain only 37.7% of the different types of compounds tested. Pearl millet inhibited the growth of *Serratia marcescens* (14.00 ± 0.882), *Salmonella typhi* (14.67 ± 0.577), *Proteus vulgaris* (25.33 ± 0.577) and *Staphylococcus epidermidis* (11.67 ± 0.577). The tests further established that *Zea mays* (maize) inhibited the growth of only *Proteus vulgaris*. The positive control inhibited the growth of all the microorganisms used, while dimethyl sulphoxide (DMSO) (negative control) did not inhibit any growth of the microorganisms used in the study. The analysis of variance showed that there was significant difference in the zones of inhibition among the microorganisms. These findings may justify the value of nutritional and therapeutic traditional foods in developing economies. This paper recommends use of indigenous cereals for food. Further research needs to be done to isolate and analyze active compounds and their structural composition and determine their pharmacological significance.

Key words: Pearl millet, maize, phytochemical, antibacterial, indigenous foods.

INTRODUCTION

The use of indigenous foods dates back to so many years before the colonization of Africa. Indigenous plants have been used as food. According to Kabuye and Ngugi (2001), there is very little demarcation between the

nutritional and medicinal values of most indigenous foods. A lot of misunderstanding has been created around these foods with many people, especially those living in urban areas terming them as unpalatable and diet

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for the poor. The claim is that these foods are eaten due to poverty and therefore, they do not find a place in the purchase list of many "civilized" families living in urban and even rural areas today (Nambiar et al., 2011; Burton et al., 1972; National Institute of Nutrition (NIN), 2003).

Indigenous foods can form key pillars of food security in developing countries. The ability for these plants to withstand long periods of drought can bring added value to those who are using these cereals as staple foods. According to Nyok et al. (2001), low levels of mortality were observed in communities that used indigenous plants for food. Most indigenous foods can be termed as nutraceuticals, foods which have both nutritional and medicinal values. 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). Extensive changes in food supply in Africa due to domination of the market with exotic foods in both rural and urban areas have led to the decline in family balanced diet and decrease in the extent in the use of traditional foods (Oniango, 2003).

According to Daniel et al. (2012), 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). According to Amarender et al. (2013) the share of pearl millet use 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, this comparative study on the antibacterial and phytochemical composition of pearl millet and maize. There is a gap of knowledge on the medicinal value of pearl millet since not much has been done to ascertain the antibacterial activity of pearl millet against a wide range of bacterial organisms.

In this study we report the antibacterial activity of *Pennisetum glaucum* (pearl millet) in comparison to *Zea mays* (maize) which is majorly used as a staple food in most countries in Africa today. In this paper we also show a comparison of the two cereals in terms of presence and absence of important pharmacological compounds which have no nutritional value but have health benefits.

MATERIALS AND METHODS

Pearl millet and maize samples were bought from different grain stores in Chiakariga in Meru county, Kenya. Ishiara is one of the

semi-arid areas Meru county. The region receives unreliable rainfalls and therefore used for cultivation of drought sustainable crops. The collected samples were thoroughly mixed and ground into fine powder and then stored in transparent polythene bags.

Extraction procedure

Using electric analytical beam balance, 100 g of the powdered samples were placed in a 500 ml conical flask. Methanol and water were added in the ratio of 9:1, respectively until the samples were completely submerged in the solvent. The mixture was then agitated for thorough mixing and kept for 24 h with frequent shaking for effective extraction of the plant components. The extract was filtered using Butchner funnel; Whatman no.1 filter paper and a vacuum and pressure pump. The filtrate was re-filtered again using the same apparatus. The solvent was evaporated using rotary vacuum evaporator (R-11) with a water bath at 40°C. The residue was obtained and stored at 4°C for future use.

Qualitative phytochemical analysis

The extracts phytochemical analysis for identification of chemical constituents was done using standard procedures with minor adjustments (Trease and Evans, 1989; Harbone, 1973; Sofowara, 1993).

1. Tannins: About 0.1 g of the extract was put in a test tube and 20 ml of distilled water was added and brought to boil. The mixture was then filtered and 0.1% of FeCl₃ was added to the filtrate and observations made. A brownish green color or a blue-black coloration indicated the presence of tannins.
2. Saponins: About 0.1 g of the extract was mixed with 5 ml of water and vigorously shaken. The formation of stable foam indicated the presence of saponins.
3. Flavonoids: About 0.1 g of the extract was added into a test tube. To the test tube 5 ml of dilute ammonia and 2 ml of concentrated sulphuric acid was added and heated for about 2 min. The appearance of a yellow color indicated the presence of flavonoids.
4. Terpenoids: About 0.1 g of the extract was taken in a clean test tube, 2 ml of chloroform was added and vigorously shaken, then evaporated to dryness. To this, 2 ml of concentrated sulphuric acid was added and heated for about 2 min. A greyish color indicated the presence of terpenoids.
5. Glycosides: About 0.1 g of the extract was mixed with 2 ml of chloroform and 2 ml of concentrated sulphuric acid was carefully added and shaken gently, then the observations were made. A red brown color indicated the presence of steroidal ring (glycone portion of glycoside)
6. Alkaloids: About 0.1 g of the extract was mixed with 1% of HCl in a test tube. The test tube was then heated gently and filtered. To the filtrate a few drops of Mayer's and Wagner's reagents were added by the side of the test tube. A resulting precipitate confirmed the presence of alkaloids.
7. Steroids: About 0.1 g of the extract was put in a test tube and 10 ml of chloroform added and filtered. Then 2 ml of the filtrate was mixed with 2 ml of a mixture of acetic acid and concentrated sulphuric acid. Bluish green ring indicated the presence of steroids.
8. Phenols: About 0.1 g of the extract was put in a test tube and treated with a few drops of 2% of FeCl₃; blue green or black coloration indicated the presence of phenols.

Bioassay study

Bacteria source and media preparation

The bacteria used in the study were commercial pure cultures from

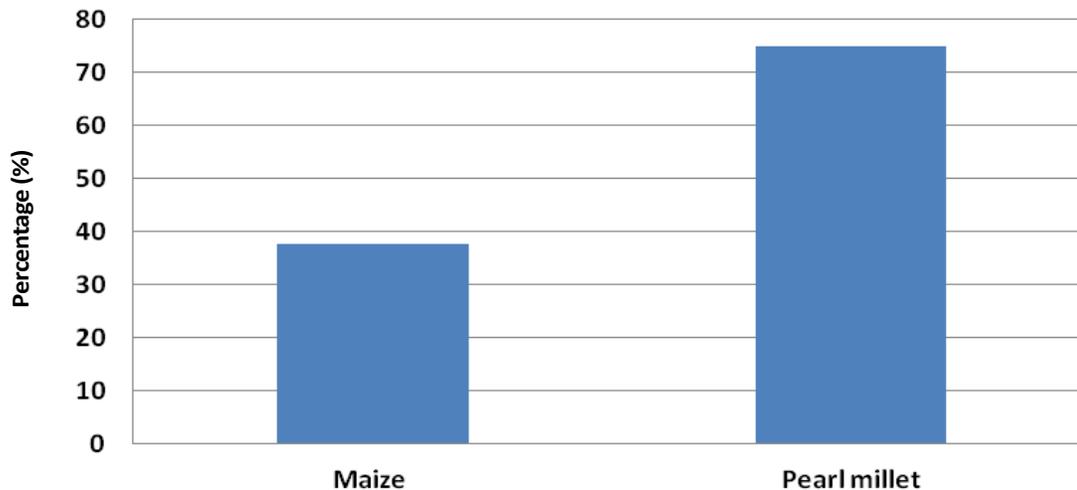


Figure 1. Comparison on the percentage of phytochemicals present in maize and pearl millet.

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 incubated at 37°C for 24 h. The blood agar media was prepared according to the manufacturer's instructions. The plates were sterilized by the use of an autoclave at 121°C. Approximately 20 ml of the prepared media was poured into the sterilized plates and then the surface of the media was flamed using a bunsen burner flame to remove air bubbles and sterilize the media surface. The 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 Biosafety cabinet wood.

Preparation of the bacterial suspension

The turbidity of each of the bacterial suspension was prepared to match to a 0.5 McFarland standard (Biruhalem et al., 2011; Donay et al., 2007). The McFarland standard was prepared by dissolving 0.5 g of BaCl₂ 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 to 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°C for 2 to 6 h until it achieved turbidity similar to the 0.5 McFarland standards. The culture that exceeded the 0.5 McFarland standard were each adjusted with the aid of a UV spectrophotometer to 0.132A° at a wavelength of 600 nm in order to obtain an approximate cell density of 1×10^8 CFU/ml.

Preparation of the extract concentrations and antibiotic

Extracts stock solutions were prepared by dissolving 500 mg in 1 ml of dimethyl sulfoxide (DMSO). An antibiotic control was made by dissolving 500 mg of penicillin in 1 ml of sterile distilled water. DMSO served as a negative control.

Determination of the bioactivity of the extract

Mueller Hinton agar plates were prepared as per the manufacturer's instructions. The media and the plates were sterilized in an

autoclave at 121°C for 15 min. The plates were flamed on the surface using a non-luminous flame to remove air bubbles and also ensure sterility of the surface. The cork borer was sterilized using a non-luminous flame. The plates and all the equipment's to be used for the experiment were then transferred into a Biosafety cabinet wood. The germicidal lamp was put on for 30 min to sterilize the surface of the plates and other equipments. The bacterial suspension was smeared on the media and five wells with a diameter of 6 mm. Each of them was drilled in each agar plate using a cork borer. Three of the wells were filled with 0.1 ml of the 500 mg/ml of the extract. The other wells were filled with 0.1 ml of 500 mg/ml of penicillin and 0.1 ml of 100% DMSO positive and negative controls, respectively. Three plates were made for each bacterial organism and extract giving a triplicate reading for each microorganism and extract. The plates were labeled on the underside and incubated at 37°C for between 24 to 48 h and the zones of inhibition measured in millimeters with the aid of a ruler.

RESULTS AND DISCUSSION

From the phytochemical analysis (Table 1), the pearl millet grains were found to contain tannins, flavonoids, terpenoids, glycosides, phenols and steroids while saponins and alkaloids were found to be absent. The maize grains were found to contain saponins, glycosides, and alkaloids but all the other compounds tested were found to be absent. The presence of these important pharmacological compounds in the grains showed how important these are in the health of a human being. However, as can be observed (Figure 1), 75% out of all phytochemicals tested were found to be present in the pearl millet grain as compared to maize. Maize contained only 37.7% of the different types of compounds tested. The high percentage of different pharmacological compounds in the pearl millet grain gives it a better edge in providing solutions to most of the health problems facing people living in the developing countries such as diarrhea, cancer and cardiovascular diseases (Asgary et

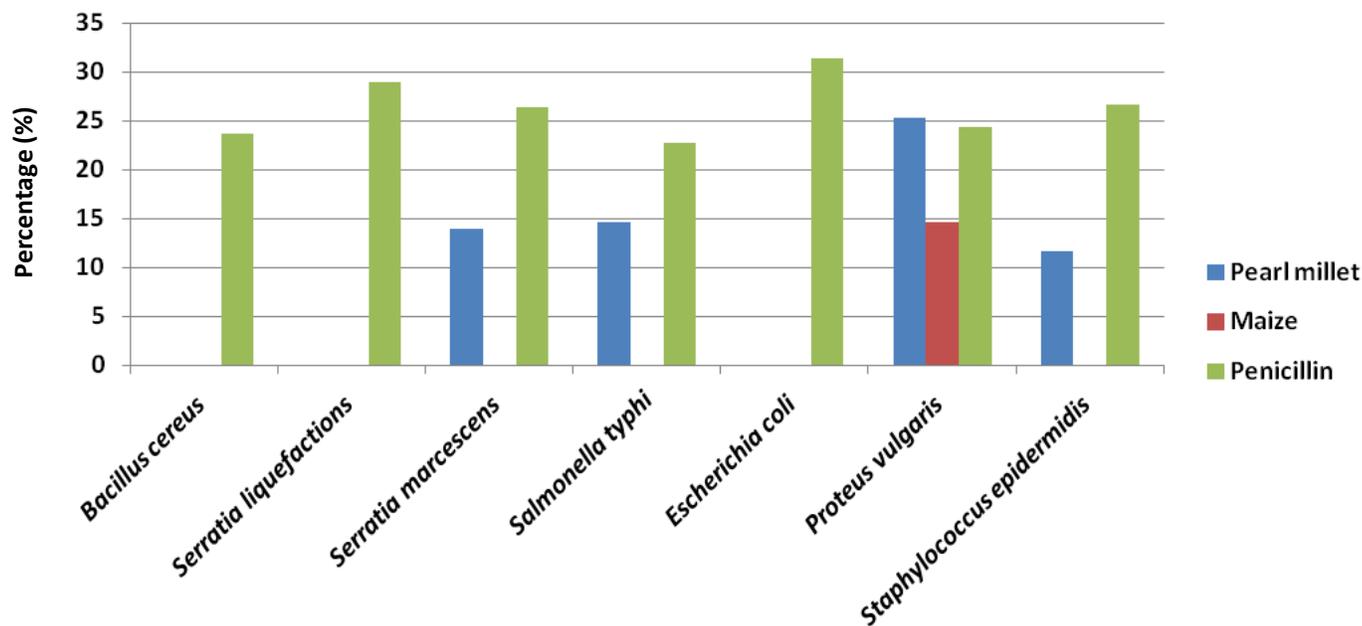


Figure 2. Comparison on the zones of inhibition of pearl millet and maize.

Table 1. Phytochemical screening of *Pennisetum glaucum* (Pearl millet) and *Zea mays* (maize).

Phytochemical	<i>Pennisetum glaucum</i> (Pearl millet)	<i>Zea mays</i> (maize)
Tannin	+++	--
Saponins	--	++
Flavonoids	+++	--
Terpenoids	++	--
Glycosides	++	+++
Alkaloids	--	++
Phenols	+++	--
Steroids	++	--

al., 2001; Kokwaro, 2009; Chung et al., 1998).

Pearl millet (Table 2) inhibited the growth of *Serratia marcescens* (14.00 ± 0.882), *Salmonella typhi* (14.67 ± 0.577), *Proteus vulgaris* (25.33 ± 0.577) and *Staphylococcus epidermidis* (11.67 ± 0.577). The pearl millet hydromethanolic extract did not inhibit the growth of *Bacillus cereus*, *S. marcescens* and *Escherichia coli*. Penicillin, which was used as the positive control, inhibited the growth of all the microorganisms used, while DMSO (negative control) did not inhibit the growth of any of the microorganisms used in the study. The analysis of variance showed that there was significant difference in the zones of inhibition among the microorganisms. From the study (Table 3), *Zea mays* (maize), inhibited the growth of only *Proteus vulgaris*.

The antibacterial activity of pearl millet (Figure 2) was found to be greatly higher as compared to maize. This

could be attributed to the high number of phytochemicals found in pearl millet over those found in maize. The high antibacterial and phytochemical content of pearl millet shows that it may be prudent for people living in East Africa to go back to the basics of their nutrition. The study is in conformity with another research in which phenols and flavonoids were found to be present in pearl millet (Vanisha et al., 2012). The study is however different since it presents a wide spectrum of phytochemicals in pearl millet and maize. The presence of a wide range of phytochemicals in pearl millet over maize may be an explanation as to why the grain has been found to have a wide range of therapeutic effects. Examples are anticancer activity, control blood pressure and control of cholesterol levels. Pearl millet has also been used to treat against severe constipation and stomach ulcers (Haung et al., 1984; Asp, 1996). According to Raschke and

Table 2. Antibacterial activity of *Pennisetum glaucum* methanolic aqua extract against selected pathogenic microorganisms.

Microorganisms	Extract	Penicillin	DMSO
	[mean ± mean S.E (mm)]	[mean ± S.E (mm)]	[mean ± S.E (mm)]
<i>Bacillus cereus</i>	0.00±0.000	23.67±0.882	0.00±0.000
<i>Serratia liquefactions</i>	0.00±0.000	29.00±0.882	0.00±0.000
<i>Serratia marcescens</i>	14.00±0.882	26.33±0.577	0.00±0.000
<i>Salmonella typhi</i>	14.67±0.577	22.67±0.882	0.00±0.000
<i>Escherichia coli</i>	0.00±0.000	31.33±0.577	0.00±0.000
<i>Proteus vulgaris</i>	25.33±0.577	24.33±0.577	0.00±0.000
<i>Staphylococcus epidermidis</i>	11.67±0.577	26.67±0.577	0.00±0.000

S.E = standard error.

Table 3. Antibacterial activity of *Zea mays* methanolic - aqua extract against selected pathogenic microorganisms.

Microorganisms	Extract	Penicillin	DMSO
	[mean ± mean S.E (mm)]	[mean ± S.E (mm)]	[mean ± S.E (mm)]
<i>Bacillus cereus</i>	0.00±0.000	23.67±0.882	0.00±0.000
<i>Serratia liquefactions</i>	0.00±0.000	29.00±0.882	0.00±0.000
<i>Serratia marcescens</i>	0.00±0.000	26.33±0.577	0.00±0.000
<i>Salmonella typhi</i>	0.00±0.000	22.67±0.882	0.00±0.000
<i>Escherichia coli</i>	0.00±0.000	31.33±0.577	0.00±0.000
<i>Proteus vulgaris</i>	14.67±0.577	24.33±0.577	0.00±0.000
<i>Staphylococcus epidermidis</i>	0.00±0.000	26.67±0.577	0.00±0.000

S.E = standard error.

Cheema (2007), the shift from the use of indigenous foods and the adaption of global food systems has led to simplification of East African cultural practices and indigenous food systems which in turn has led to increase in non-communicable diseases in the entire region.

From this study, it can be seen that pearl millet had greater antibacterial activity over maize which can be attributed to the wide spectrum of phytochemicals found in the pearl millet grain. This clearly indicates the reason as to why it may be prudent to formulate food systems which encourage use of indigenous foods (in this case pearl millet) as the basic staple food in Africa and other developing countries. The antibacterial activity of the cereal could be due to synergistic effect of two or more compounds in them. The data obtained in this research may be a scientific justification on the therapeutic value of pearl millet, contrary to the current trend in which replacement of indigenous foods with maize has been quickly growing, especially in East and Central Africa (Johns and Sthapit, 2004). More research could be conducted in future to determine if pearl millet could be used to treat against gastro-intestinal infections and ulcers

(Goodwin et al., 1971; Neter, 1943; Hallstrom and McCormick, 2011).

The data obtained and reported in this study may be a scientific justification of the great nutritional and therapeutic value of traditional foods in Kenya and Eastern Africa as a whole. However, further research needs to be done to isolate the active compounds, analyze their structural composition and their entire pharmacological value. From the results of this study, it is essential to encourage people to incorporate pearl millet in their daily diets to begin enjoying some of these great nutritional benefits of this very nutritious indigenous cereal.

Conflict of interest

The authors declare no conflict of interest.

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