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ANTIBACTERIAL ACTIVITY OF FINGER MILLET (*ELEUSINE CORACANA*) ON SOME SELECTED CLINICAL BACTERIA.

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phytochemical, antimicrobial, finger millet, bacteria, zone of inhibition

Abstract

Finger millet in northern Nigeria was subjected to phytochemical screening using standard procedures. The agar well method was used to test the antibacterial activities of methanolic and aqeous (combined) extracts of the grain on Salmonella typhi, Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus. The result of the antimicrobial activity as indicated by zone of inhibition ranged from 1-8mm for different extract concentrations. The finger millet extract showed zones of inhibition of 8mm against Pseudomonas aeruginosa at a concentration of 100mg/ml, 3mm at 50mg/ml and 2mm at 25mg/ml concentrations. The inhibition zones of Escherichia coli at extract concentrations of 100mg/ml,50mg/ml,25mg/ml, 12.25mg/ml and 6.125mg/ml were 4mm, 3mm, 3mm, 6mm and 1mm respectively, and for Staphylococcus aureus were 5mm,2mm, 1mm at 100mg/ml, 50mg/ml and 12.25mg/ml respectively. The zones of inhibition against all the tested isolates at 100mg/ml was not significantly different from those of 50mg/ml (p=0.160), 25mg/ml (p=0.067) and 12.5mg/ml (p=0.160), but significantly higher than 6.125mg/ml (p=0.05). The results further showed that E. coli, S. aureus and

P. aeruginosa did not differ significantly in their susceptibility to the varying concentrations of the plant extract (p=0.229). Although S. aureus and S. typhi also did not differ significantly in their susceptibility to the varying concentrations of the extract (p=0.157), but susceptibility by S. typhi was significantly lower than those of E. coli (p=0.007) and P. aeruginosa (p=0.015). The qualitative phytochemical analysis indicated the presence tannin/phenol, flavonoids, alkaloid, saponin, glycosides, terpenoid and steroids in finger millet. The proximate analysis revealed the moisture (9.98%), ash (2.81%), protein (1.45%), fat (1.67%), carbohydrate (78.08%) and energy contents (357.18kcal). The quantitative phytochemical revealed total phenolic content (6.57 mg/100g) and total flavonoid content (0.224 mg/100g). The overall results indicate that finger millet are potent antimicrobial preparations at least invitro and also have high nutritional value.

INTRODUCTION

Finger millet (*Eleusine corocana*) is a small cereal grain grown in the semi-arid sub-tropical regions of Africa and Asia where it is one of the cereal staples (ICRISAT/FAO 1996; Obilana and Mayasa, 2002). It is cultivated mainly in eastern and central and southern Africa, and in Asia it is produced in India, Nepal and China. Finger millet annual world production is at least 4.5 million tonnes (House, 1995; Obilana and Manyasa, 2002). The prevailing high temperature and relative humidity in the tropics favour growth of micro-organisms (particularly fungi) and cereal grains (Siwela *et al.*, 2007).

According to US National Research Council (1995), finger millet has many good qualities. It is adapted to various agro-climatic conditions, its seed can be stored for several years without insect damage and it is one of the most nutritious cereal grains.

Finger millet has gained importance because of its nutritional quality in terms of dietary fibre, functional fibre, starch pattern as well as high calcium and iron contents (Karki and Kharel, 2012).

Malt from finger millet is extensively used in various supplementary food formulations (Malleshi, 2005).

Finger millet grain contains various phenol compounds including tannin (Dykes and Rooney 2006) that have been shown to contribute to its antioxidant properties (Sripriya *et al.,* 1996). Some finger millet grain types contain tannin as sorghum (Dykes and Rooney, 2006) and may contain flavonoid compound that are similar to those found in sorghum.

Finger millet is reported to have anti-ulcerative properties and has been used to lower glucose and cholesterol in the diet of diabetic rat models. Its seed coat matter is a rich source of dietary fibre and its phenolic compounds have been found to exhibit blood glucose and cholesterol lowering, nephro-protective and anti-catactogenic properties. The *invitro* inhibition of snake venom phospholipases by finger millet phenolics have also been reported (Methangi and Sudha, 2012). Despite its importance, finger millet is grossly neglected both scientifically and internationally (US National Research Council, 1996).

Plant phenolics are currently receiving a lot of attention as they show potential health promoting effects which are attributed to their antioxidant activity (Scalbert *et al.,* 2005). Tannin has been shown to exhibit higher activity than other phenolics (Hagerman *et al*., 1998).

Currently there are limited data on the phenolic composition of finger millet grains; the relationship between grain characteristics e.g. color and phenolics content and composition are hardly known (House, 1995).

Because of the high rate of consumption by the people, phytochemical and antimicrobial property of finger millet grain is important so as to ascertain its health benefits.

The aim of this study is to analyze the phytochemical and antimicrobial activity of finger millet on some selected clinical bacteria as well as determine its nutritional value.

MATERIALS AND METHODS

Sample Collection and Preparation

Finger millet was purchased from a local market in Kamaru Chawai, Kauru local government area of Kaduna state assuring the best quality. The sample was washed to remove the debris and dried in a hot air oven at 30°C – 40°C

for 48hrs and ground into powder. The powdered grain was used for the analysis.

Preparation of Plant Extracts

Methanol extraction

One liter of 80% methanol extraction fluid was mixed with 200g of powdered sample material. The mixture was kept for two days in tightly sealed vessels at room temperature. It was then filtered through muslin cloth. Further extraction of the residue was repeated 3-5 times until a clear colorless supernatant extraction liquid was obtained indicating that no more extraction from the powdered sample was possible. The extracted liquid was subjected to water bath temperature at 40°C. This was kept under a ceiling fan to dry. The extract was weighed and portion of it was used for phytochemical screening while the rest was used for the susceptibility test. (Hena *et al.,* 2010).

Aqueous Extraction (Cold Water)

40g of the powdered extract was soaked in 300ml of distilled water for 48hours at room temperature. The extract was filtered and evaporated in water bath at 80°C to concentrate the extract, and the extract was finally stored in sterile bijou bottle and labeled accordingly (Effriam *et al.,* 2001).

Phytochemical Screenings

Standard phytochemical test was carried out on the seed sample using the method of Sofowora (2003) to determine the presence of alkaloid flavonoids, glycosides, saponin, tanins, anthraquinones, steroids, terpenoids.

Test for Phenol and Tannin

Aqueous extract was mixed with 2ml of 2% solution of $FeCl_3$. A blue green or black colouration indicated the presence of phenol and tannins.

Test for Flavonoid (Shinoda test)

5ml of the extract was mixed with 1.5ml of 50% methanol and warmed on steam bath. Metallic magnesium and 5drop of concentrated hydrolic acid

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were added. A red or orange colour indicates the presence of flavonoids aglycone.

Test for Alkanoid (Dragendoff's test)

To about 5ml of extract, 1% diluted HCl (20ml) was added in a conical flask, heated on a steam bath and filtered. The filtrate was made alkaline with 28% NH_3 solution and then extracted with chloroform ($3\times5cm^3$). The combine $CHCl_3$ extracts were concentrated and treated with equal volume of 1% Hcl. Dragendoff's reagent (2ml) were added and occurrence of orange red precipitate indicated the presence of alkanoid.

Test for Saponin

5ml extract was mixed with 5ml of distilled water in a test tube and it was shaken vigorously, the formation of stable foam was taken as an indication for the presence of saponin.

Test for Glycoside

5ml of the extract was mixed with 2ml of glacial acetic acid containing 1-2 drops of 2 % solution of $FeCl_3$. The mixture was then poured into another tube containing 2ml of concentrated H_2So_4. A brown ring at the interface indicated the presence of cardiac glycosides.

Test for Terpenoid

Extract was dissolved in 2ml of chloroform and evaporated to dryness. To this 2ml of concentrated H_2SO_4 was added and heated for about 2minutes. A grayish colour indicates the presence of terpenoids. **Test for Steroid**

Extract was mixed with 2ml of chloroform and concentrated H_2SO_4 was added sidewise. A red colour produced in the lower chloroform layer indicates the presence of steroid. Another test was performed by mixing crude extract with 2ml of chloroform, the 2ml of each concentrated H_2SO_4 and acetic acid was poured into the mixture, and the development of a grayish colouration indicates the presence of steroids.

Test for Anthraquinone

Small portion of the extract was shaken with 10ml of benzene and filtered. 5ml of 10% NH_3 was added to the filtrate and stirred. The production of a pink-red or violet colour indicates the presence of free anthraquinones.

Isolation and identification of test Organism

The test organisms used for the study were obtained from the microbiology laboratory of Abubakar Tafawa Balewa University Teaching Hospital Bauchi, Bauchi state. The organisms are *Pseudomonas aeruginosa, Salmonella typhi, Escherichia coli* and *Staphylococcus aureus.* The following biochemical tests were carried out to confirm and authenticate the organisms: Gram stain, Catalase, coagulase, Oxidase, Indole, sugar fermentation, methyl red, Voges Proskauer and Hydrogen Sulphide Production (Cheesbrough, 2004).

Antimicrobial Susceptibility Test

Inhibition of microbial growth was tested by using the well method as described by Drago *et al.,* (1999). Standard aseptic microbial methods were followed.

Mc Farland Standard

0.5 Mc Farland standard was prepared by adding 0.5ml of 1% Barium Chloride ($BaCl_2$) to 99.5ml of 1% sulphuric acid (H_2SO_4) solution. The turbidity standard was used for the estimation of the amount of bacteria in both cultures (culture for 24hr at 37^0C) to pour into 5ml of distilled water in order to obtain standard bacteria concentration of 1×10^8 bacteria cells (Bauer *et al.,* 2003).

Serial Dilution

Stock solution was prepared by using 1g of the solid plant extract dissolved in 10mg/ml. serial doubling dilution was employed to obtain concentration of 100mg/ml, 50mg/ml, 25mg/ml, 12.25mg/ml, and 6.125mg/ml respectively. (Bauer *et al.,* 2003).

Proximate Analysis

To be so sure of the content of the seed and that of the seed coat before any analysis, proximate determination was performed on small portion of sample in terms of percentage dry matter protein, lipid content, ash, moisture, carbohydrate content and energy (AOAC, 2003).

Determination of Total Phenols and Flavonoid Content by Spectrophotometric Method

The fat free sample was boiled with 50ml of ether for the extraction of the phenolic component for 15minutes. 5ml of the extract was pipetted into a 50ml flask, and then 10ml of distilled water was added. 2ml of ammonium hydroxide solution and 5ml of concentrated amyl-alcohol were also added. The samples were made up to mark and left to react for 30minutes for colour development. This was measured at 505nm. (Edioga and Gomina, 2000).

For the determination of total flavonoid content, the samples were extracted repeatedly with 100ml of 80% of aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No. 42. The filtrate was later transferred into crucible and evaporated into dryness over a water bath and weighed to a constant weight. (Bohm and Kocipai- Abyazan, 1994).

Statistical analysis

ANOVA was used to test for significance difference in all the data obtained from zones of inhibition. All statistical analyses were carried out using the SPSS 17.0 window based program. Significance difference and non-significance difference was defined when $p \leq 0.05$ and $p > 0.05$ respectively.

RESULT

The result of phytochemical screening of finger millet on aqeous and methanolic extract were as presented in table 1. The result revealed the presence of medically active compounds in the extract such as tannin, phenol, glycosides, flavonoids, terpernoids in both methanolic and aqeous

extracts. Alkaloids and steroids were absent in the aqeous extract while saponin was absent in methanolic extract. Anthraquinones and phlobatannins were absent in both aqueous and methanolic extracts.

Table 2 summarizes the phytochemical characteristics of finger millet at different concentrations of 100mg/ml, 50mg/ml, 25mg/ml, 12.25mg/ml and 6.125mg/ml. The result reveals the presence of active compounds at different concentration. Tannin, phenol and flavonoid were present in all the concentrations while steroid, alkaloids and anthraquinone were absent in all the concentrations. From the table, it could be seen that saponin and tarpenoids are present even in lower concentrations.

Table 3 shows that the grain's aqueous and methanol (combined) extract exhibited remarkable antibacterial activity against some of the test organisms (*Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus*) with zones of inhibition within the range of 1-8mm.The antibacterial activities of the grain extract against *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* were all inhibited at different concentration except *Salmonella typhi* which was not inhibited even at the highest concentration of 100mg/ml.

The proximate analysis of the finger millet revealed moisture, ash, fat, protein and carbohydrate contents to be 9.98%, 2.8132%, 1.6700%, 7.4505% and 78.00863% respectively. The energy content was 357.1772 kcal (Table 4). The quantitative determination of total phenolic and flavonoid content reveals 6.57mg/100g and 0.224mg/100g respectively (Table 5).

The zones of inhibition against all the tested isolates at 100mg/ml was not significantly different from those of 50mg/ml ($p=0.160$), 25mg/ml ($p=0.067$) and 12.5mg/ml ($p=0.160$), but significantly higher than 6.125mg/ml ($p=0.05$). The results further showed that *E. coli, S. aureus* and *P. aeruginosa* did not differ significantly in their susceptibility to the varying concentrations of the plant extract ($p=0.229$). Although *S. aureus* and *S. typhi* also did not differ significantly in their susceptibility to the varying concentrations of the extract ($p=0.157$), but susceptibility by *S. typhi* was significantly lower than those of *E. coli* ($p=0.007$) and *P. aeruginosa* ($p=0.015$).

Table 1: Results of Phytochemical screening of finger millet using aqueous and methanolic extract.

	Extract	
Phytochemical properties	***Aqeuos***	***Methanolic***
Tanin/Phenol	++	+++
Flavonoids	++	+++
Alkaloids	-	+
Saponins	+	-
Glycoside	++	+
Terpenoids	+	+
Steroids	-	+
Anthraquinones	-	-
Phlabatannin	-	-

***Key** : +++ = highly positive, ++ = moderately positive, + = slightly positive, - = negative*

Table 2 Results of Phytochemical screening of methanolic extract of finger millet at different concentrations.

	Extract concentration (mg/ml)				
Phytochemical Properties	***100***	***50***	***25***	***12.25***	***6.125***
Tanin/Phenol	+++	+++	+	+	+
Flavonoid	++	++	+	+	+
Alkaloids	-	-	-	-	-
Glycosides	+++	++	++	++	+
Terpenoid	++	+	+	-	-
Steroids	-	-	-	-	-
Anthraquinone	-	-	-	-	-
Phlabotannin	-	-	-	-	-
Saponin	++	++	+	+	-

Keys: +++ = highly positive, ++ = moderately positive, + = slightly positive, - = negative, mg/ml = milligram/milliliter.

Table 3: Antibacterial activity of finger millet aqueous and methanol (combined) extracts on some selected microorganism using different concentrations.

	Zone of inhibition (mm) at varying concentration (mg/m)				
Test micro organism	***100mg/ml***	***50mg/ml***	***25mg/ml***	***12.5mg/ml***	***6.125mg/ml***
Escherichia coli	4(++)	3(++)	3(++)	6(+++)	1(+)
Staphylococcus aureus	5(++)	2(+)	0(-)	1(+)	1(+)

Pseudomonas aeruginosa	*8(+++)*	*3(++)*	*2(+)*	*1(+)*	*2(+)*
Salmonella typhi	*0(-)*	*0(-)*	*0(-)*	*0(-)*	*0(-)*

Zone of inhibition (mm) at varying

Key : - = no zone of inhibition, mg/ml= milligram per milliliter, + = low zone of inhibition (1.0-2.9mm), ++ = moderate zone of inhibition (3.0-5.9mm), +++ = high zone of inhibition (6.0-9.0mm).

Table 4. The Proximate analysis of the Finger Millet Proximate content percentage(%) kilo cals (kcal)

Proximate content	*percentage(%)*	*kilo cals (kcal)*
Moisture	*9.98*	-
Ash	*2.81*	-
Fat	*1.67*	-
Protein	*1.45*	-
Carbohydrate	*78.08*	-
Energy	-	*357.18*

Table 5: Determination of Quantitative Phenolic and Flavonoid content of finger millet

Sample	*Total Flavonoid(mg/100g)*	*Total Phenol (mg/100g)*
Finger Millet	*0.224*	*6.57*

DISCUSSION

The phytochemical study carried out on finger millet aqeous and methanolic extracts revealed the presence of phenol, tannin, flavonoid, saponin, glycoside, steroid and terpenoid, which contribute to the antimicrobial activities on the bacteria used in the study. This is similar to the findings of Siwela *et al.,* (2010) that finger millet extract from the seed coat showed high antibacterial and antifungal activity compared to the whole flour extract. Phytochemical screening conducted on finger millet grain extract revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities (Sofowora, 1993).

The analysis carried out on the finger millet extract shows that the higher the concentration of the extract the more the inhibition on the growth of

Staphylococcus aureus and *Pseudomonas aeruginosa*, with no growth on *Salmonella typhi*. This agrees with the findings of Mathangi and Sudha (2012) that *Salmonella spp* can only be suppressed by fermented finger millet.

The analysis carried out on the nutritional value revealed high nutritional value and energy content of the grain. This agrees with the findings of Karki and Kharel, (2012) that finger millet grain has gained importance because of its nutritional quality.

The carbohydrate content (78.08%) of finger millet in this study is similar with the findings of work carried out in the Pretoria by Mc Donough, (2000) that carbohydrate make up 70-76% of the finger millet and agree with the findings of work carried out in India by Bhatt *et al*., (2003) which reveals that the total carbohydrate content of finger millet is in the range of 72-79.5%.

The phenolic and the flavonoid content present in finger millet grain contribute to its antimicrobial activity as earlier stated by Viswanath *et al*., (2009) that seed coat phenolic extract is active against fungi and bacteria. The total phenolic content in this study (6.57mg/100g) shows great disparity with the findings of Viswanath *et al.,*(2009) who reported that the seed coat of finger millet had the highest phenolic content (12.60mg/100g), followed by whole grain flour (7.30mg/g) and also similar with the other flour fractions which had much lower phenolic content (3.30 to 4.30mg/100g) than that of the seed coat; and this is similar with the findings of Thippeswamy and Naidu, (2005) that the phenolic content of germinated finger millet had significant increase which was found to be 7.52mg/100g.

CONCLUSION

The overall result indicate that finger millet are potent antimicrobial preparation at least *in-vitro.* This lays credence to the use of finger millet in the treatment of various infectious diseases. The medically active ingredients of the grain extracted were able to inhibit the growth of organisms. Also the nutritional value of finger millet grain was analyzed and found to contain high nutritional value.

Recommendation

Further studies on antifungal, antiviral and other antimicrobial activities of finger millet could be carried out.

Comparative study on the active ingredient of the seed coat and the endosperm could also be useful in the study of finger millet.

Since finger millet is of both medical and food importance, it is recommended that it should be a staple food and the seed coat should not be discarded during food processing, hence it contains active ingredient.

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