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Full Length Research Paper

Evaluation of the antimicrobial activity of saponins extract of *Sorghum Bicolor* L. Moench

Soetan k. O.[1]*, Oyekunle M. A.[2], Aiyelaagbe O. O.[3] and Fafunso M. A.[4]

[1]Department of Veterinary Physiology, Biochemistry and Pharmacology, University of Ibadan, Ibadan,
[2]Department of Veterinary Microbiology and Parasitology, University of Agriculture, Abeokuta.
[3]Department of Chemistry, University of Ibadan, Ibadan.
[4]Department of Biochemistry, University of Ibadan, Ibadan.

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The n-butanol purified saponin extract of sorghum bicolor were screened for anti-bacterial activity against three pathogenic microbes; *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*. The extract inhibited the growth of the *S. aureus*. It was concluded that the saponins have inhibitory effect on gram-positive organism but not on gram negative organism and the fungi.

Key words*: Sorghum bicolor*, saponins, antimicrobial activity.

INTRODUCTION

Sorghum, which belongs to the family Graminae, is the fourth most important cereal crop after wheat, rice and maize. It is cultivated in different parts of the world and it has formed a part of the farming system of the people in these ecologies (Lukhele, 1981). Sorghum is considered to be the fifth most extensively grown crop in the world and the third largest crop harvest in the USA (Kazanas and Fields, 1981).

Saponins are glycosides occurring widely in plants. They are abundant in many foods consumed by animals and man (Cheeke, 1971). Saponins are divided into two groups: steroidal saponins which occur as glycosides in certain pasture plants such as *Brachiaria decumbens* and *Panicum* sp., and triterpenoid saponins, which occur in soybeans and alfalfa. Many pharmacological activities have been reported about saponins such as antibiotic, antifungal, antiviral, hepatoprotective anti-inflammatory and anti-ulcer (Oakenfull and Fenwick, 1981; Price and Fenwick, 1990; Just et al., 1998, Chao et al., 1998; Tschesche and Wulff, 1973; Jun et al., 1989; Okubo et al., 1994; Arao et al., 1998; Zhang and Hu, 1985).

The development of resistant microorganisms on prolonged exposure to existing antimicrobial agents has been known for a long time (Weisser et al., 1966). This has led to the continual search for ways of eradicating resistant strains of micro organisms.

Many bioassay reports have indicated the presence of antimicrobial compounds among many higher plants in Nigeria (Ogunlana and Ramstad, 1975; Durodola, 1980). There are also many reports of chemical investigations which have identified various chemical compounds responsible for the antimicrobial activities of ethnomedicinal plants (Obasi and Igbochi, 1992; Ahmad et al., 1986; Nakhala et al., 1980). However, most of these investigations were conducted on herbal plants. There is little or no scientific information concerning the antimicrobial activity of food plants in Nigeria.

Antimicrobial drug resistance in bacterial pathogens is of national and international concern. Therefore, in this study we determined the concentration of saponins in *Sorghum bicolor* and its antimicrobial activities.

MATERIALS AND METHODS

Plant material

Sorghum bicolor was bought at Bodija market, Ibadan and authenticated by an Agronomist at the Department of Agronomy, University of Ibadan, and Ibadan.

Preparation of crude saponin extracts

The sorghum seeds were sun dried. The dried seeds were ground

*Corresponding authors E-mail soetangboye@yahoo.com.

Table 1. Antibacterial and antifungal sensitivity test of *S. bicolor* saponins.

Concentrations (mg/ml)	100	50	25	12.5	6.2	3.0	1.5	Control	Result
MIC of *S. bicolor* saponins on *E. coli*	+	+	+	+	+	+	+	+	Resistant
MIC of streptomycin on *E. coli*	-	-	-	-	-	+	+	+	Sensitive at 6.25 mg/ml
MIC of *S. bicolor* saponins on *Staphylococcus aureus*	-	-	-	+	+	+	+	+	Sensitive at 25 mg/ml
MIC of penicillin on *Staphylococcus aureus*	-	-	-	+	+	+	+	+	Sensitive at 25 mg/ml
Candida albicans	+	+	+	+	+	+	+	+	Resistant

once using electronic grinder. 900 g of sorghum seeds were exhaustively separated for 10 h in a Soxhlet extractor using hexane (boiling range 68-69ºC).This removed the lipids and other pigments (Fenwick et al., 1992). The solvent was changed to methanol (boiling range 64-65.5ºC) and the extraction was continued for the next 12 h. This removed the saponins, together with low molecular weight substances such as sugars, the phenolic compounds, oligosaccharides and flavonoids (Fenwick et al., 1992). The resulting solutions were evaporated to dryness to yield 32 g methanolic extracts. The presence of saponins in these methanolic extracts was detected by the characteristic frothing tests and thin layer chromatography.

In order to obtain partially purified saponins the methanolic extracts were loaded on to a column of RP-18 powder (Octadecyl slane bonded to silica gel particle size 15-25 YM (JT Baker, Germany). The column was washed with water to remove the sugars and oligosaccharides while further elution with 30% methanol (v/v) removed the flavonoid compounds and other phenolic compounds. Subsequent elution with 100% methanol removed the saponins (Fenwick et al., 1992; Igile, 1995). This yielded 24 g of crude saponins. The saponin extracts were dispensed into clean sterile bottles and stored in the refrigerator at –4°C until needed.

The saponins extracts were subjected to thin-layer chromatography (TLC) on silical gel plates (0.25 mm silica gel) using the solvent system methanol/distilled water (4:1). The developed plates were dried at room temperature. Visualization of saponin on developed plates was done by spraying with 50% (v/v) sulphuric acid. The sprayed chromatograph were allowed to dry for 15 min at room temperature and then heated at 105ºC for 3 min in oven until the colour developed reached its maximum.

Frothing test was done on the basis that aqueous solutions of saponins form very stable foams. 1 ml of the concentrated methanolic solution of the extract was shaken with 5 ml of distilled water in a test tube. Formation of stable foams would indicate the presence of saponins.

Antimicrobial activity determination

Sensitive microorganisms employed in this study were *Escherichia coli* NCTC 10418 (Gram negative), *Staphylococcus aureus* (Gram positive), and *Candida albicans* (a fungus), all of which were obtained from the Department of Veterinary Microbiology and Parasitology, University of Ibadan, Nigeria. The two bacteria and the fungus were recovered by overnight growth at 37°C on nutrient and Sabourand's agar (Oxoid Laboratory,England: pH 7.4) plates, respectively.

To determination of the Minimum Inhibitory Concentration (MIC) of sorghum saponins extract, 8 sterile capped tubes of 7×1.3 cm were arranged in a row, with one row for each organism listed above. 1000 mg sorghum saponins extract was diluted in 10 ml of 0.1% peptone water (tryptone soya broth for the fungus) to obtain a working solution of 100 mg/ml of saponins. Serial dilutions of the working solution were made. 1 drop of $^{1}/_{100}{}^{th}$ overnight broth culture of each organism was delivered into each tube and incubated for 18 to 24 h at 37ºC. The sensitivity at the highest dilution of each row was read off where opalescence rather than turbidity was noticed. Penicillin and streptomycin antibiotics were used as standards for the bacteria.

RESULTS AND DISCUSSION

On the basis of the efficiency of extraction-purification, the amount of saponins in *S. bicolor* was found to be 2.7%. A combination of frothing test and thin layer chromatography showed that saponins were present in the 100% methanolic eluates only. Thin-layer chromatography reveals one spot attributable to saponin. Stable foams were observed in the frothing test indicating the presence of saponins. The saponin extract inhibited the *S. aureus* in concentration of 50 and 25 mg/ml. The MIC of streptomycin for *E. coli* was 6.2 mg/ml while the MIC of penicillin for *S. aureus* was 25 mg/ml (Table 1). This shows that sorghum saponins have effect on Gram +ve bacteria but not on Gram –ve bacteria and fungi.

The results of the present study show that saponin is present in *S. bicolor* in some significant amounts. This is similar to reports by Pariza (1996) and Salunkha et al. (1990) that cereals and other plant foods may contain significant amounts of toxic or anti-nutritional substances. The results also show that the crude saponin extracts prepared from *S. bicolor* posses antimicrobial property. The saponin extracts were inhibitory to the growth of the Gram-positive bacteria used in the test but were not inhibitory to the growth of the Gram-negative bacteria and fungi used. This is not surprising because the Gram-negative bacteria and fungi have been shown to be more resistant to antibiotics (Hugo and Russell, 1983). It is interesting that the crude saponins were active against *S. aureus,* a pathogen which has been implicated in several infections of human and animals. It is equally interesting to note that the crude saponins compared favourably with the standard antibiotics such as penicillin, which were used in this study. In addition, the saponin extract is a mixture of several saponins each of which could be as effective as or even more effective than the popular

antibiotics currently being used after purification.

The ineffectiveness of the saponins from *S. bicolor* on Gram-negative bacteria such *E. coli* and fungus such as *C. albicans*, may be as a result of the protective effect of the microbial coats. The saponins may not be able to penetrate the cell membranes of the microorganisms (Soetan, 2003). Tchesche and Wulff (1973) confirmed the weak antibacterial and fungistatic effects of the majority of saponins. Also Farnsworth (1966) concluded that higher or lower antibiotic effectivity is typical of all saponins. All these might account for the inability of the saponin extract to inhibit the growth of the Gram-negative bacteria and the fungus used in this study.

The present study has revealed that saponin is present in *S. bicolor*, a popular cereal consumed worldwide. It also revealed that the crude saponin extract from *S. bicolor* has useful antimicrobial properties. This finding is consistent with previous published reports that specific saponins could have antimicrobial properties (Fenwick et al., 1992; Campbell, 1993).

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