Research Journal of Microbiology

ISSN 1816-4935

Academic
Journals Inc.

www.academicjournals.com

Research Journal of Microbiology 2 (3): 284-288, 2007
ISSN 1816-4935

Quantitative Determination of Tannin Content in Some Sorghum Cultivars and Evaluation of its Antimicrobial Activity

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Abstract: In the present study, tannin content was investigated in three Sudanese sorghum cultivars. In addition, the inhibitory effect of natural tannins isolated from sorghum grains as well as that of commercial tannins was detected against *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhimurium* (bacteria), *Aspergillus niger*, *Aspergillus flavous* (mould) and *Saccharumyces cerivisae* (yeast). The results indicated that natural tannin from sorghum has a notable antimicrobial activity against most of the examined microorganisms, the higher antimicrobial activity among all examined organisms was found against *Salmonella typhymurium* and *Saccharomyces cerivisae*. The results also indicated that commercial tannins are more effective than natural tannins. This study has shown the importance of commercial tannins and sorghum tannins as antimicrobial and preservative agents.

Key words: Tannin, phenolic compounds, *Tabat*, *Dabar*, *Feterita* sorghum, antimicrobial activity

INTRODUCTION

Sorghum is not only a staple cereal for millions of poor farmers in the world, but it has also good feed and forage value. Sorghum grain is used in the manufacture of different items such as wax, starch, alcohol, dextrose sugar and edible oil.

In Sudan sorghum is the most important cereal crop in terms of acreage and production and a human food. Sorghum improvement in Sudan started in 1918 (Mahmoud, 1978). A good collection of local and introduced sorghum types were made and studied by Evelyn in the early forties. Since early sixties and up to date a lot of work has been done on genetic improvement of sorghum and production of *Tabat* and *Dabar* sorghum cultivars are good examples of this improvement.

Tannins are phenolic compounds that precipitate protein. They are composed of a very diverse group of oligomers and polymers (Palo, 1985; Waghorn *et al*., 1990). The presence and consequent interaction of tannins and proteins in the seeds of cereals and legumes have been believed to be of the factors involved in reduced protein digestibility (Bressani *et al*., 1983; Gupta, 1987). Most berries, such as cranberries and blueberries contain both hydrolysable and condensed tannins (Vattem *et al*., 2005; Puupponen-Pimiä *et al*., 2001).

Tannins are important ingredient in the process of tannin leather. Oak bark has traditionally been the primary source of tannery tannin, though synthetic tanning are also in use today.

The objectives of the present study were to quantify tannin content in some sorghum grains samples as well as detection of its antimicrobial activity.

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MATERIALS AND METHODS

The Plant Material

Sorghum seeds samples from three cultivars of sorghum were used in the present study; these cultivars included *Tabat*, *Dabar* and *Feterita*. The first two cultivars were obtained from Wad- medani local market, while the third cultivar was obtained from Gedarif local market (May, 2006). All grain samples were freed from foreign materials like stones, sand and dust. The seeds were then washed with water, dried and milled into fine flour using a laboratory mill (Christ and Norris Limited, England).

Methods

Determination of Tannins Content

The tannin contents were determined using Folin Denis Reagent as described by Makkar *et al*. (1993). In that method, a standard calibration curve was prepared and the Absorbance (A) against concentration of tannins at specific wave length was estimated as follows:

Suitable aliquots of the tannin-containing extract (initially: 0.05, 0.2 and 0.5 mL) were pipetted in test tubes, the volume was made up to 1.00 mL with distilled water, then 2.5 mL of sodium carbonate reagent were added. Then the tubes were shaken and the absorbance was recorded at 725 nm after 40 min. The amount of total phenols was calculated as tannic acid equivalent from the standard curve.

Testing of Tannic Acid and Natural Tannins for Antibacterial Activity

Tannic acid at 0.5, 10. 20, 2.5 and 25 g L^{-1} concentrations was dissolved in distilled water and tested against *Staphylococcus aureus* (ATCC 25923), *Esherichia coli* (ATCC 25922), *Salmonella typhimurum* (ATCC 14028) (bacteria)and mould (*Aspergillus niger* and *Aspergillus flavous*-locally isolated strains) and one species of yeast (*Saccharomyces cervisae*-commercial strain). The examined microorganisms were obtained from the Medical laboratory-University of Gezira. The examined microorganisms were thoroughly mixed with 200 mL of sterile molten nutrient agar, which was maintained at 45°C. Twenty milliliter aliquots of the inoculated agar were distributed into sterile Petri dishes. The agar was left to set and each of these plates were cut using a sterile cork borer and the agar discs were removed. Alternate cups were filled with 0.1 mL sample of tannic acid, extracted tannins using adjustable pipette and allowed to diffuse at room temp for 2 h. The plates were then incubated in the up right position at 37°C for 10 h; 2 replicates were carried out for the tannic acid, extracted tannins against each of tested organisms. After incubation, the diameter of the resultant growth inhibition zones was measured; averaged and mean values were tabulated as described by Bary *et al*. (1970) and Cruickshank *et al*. (1975).

The results were interpreted in term of the commonly used terms (sensitive) and (resistant). Concentration of the tannic acid used was 10-20 g L^{-1} dissolved in sterile distilled water. Concentration of extracted tannins used were (1:10) dissolved in methanol.

Testing of Tannic Acid and Natural Tannins for Antifungal Activity

For antifungal activity the same method for bacteria was adopted using sabouraud dextrose agar instead of nutrient agar. The fungal culture was maintained on sabouraud dextrose agar and incubated at 25°C for seven days. The fungal growth was harvested and washed with sterile distilled water and finally suspended on 100 mL sterile distilled water and the suspension was stored in a refrigerator till used.

RESULTS AND DISCUSSION

Figure 1 shows the concentration of tannins in the different sorghum samples. High levels of tannin were found in *Dabar* sorghum compared with the other sorghum cultivars samples. However, these findings were in agreement with those of Harris and Burns (1970) who indicated that the seed coat colour of sorghum grain was associated with the tannin content; the brown varieties contained more tannin. In contrast, Bullard *et al*. (1980) found insignificant correlation between tannin content and seed coat colour. While, Blession *et al*. (1963) indicated the contribution of non-tannin compounds to the pigmentation of the seed coat. On the other hand, *Feterita* sorghum also contained high concentration of tannin but less than that of *Tabat*.

Antibacterial Activity of Tannic Acid and Natural Tannins

Tannic acid and natural tannins showed the higher antibacterial activity against *Salmonella typhimurium*, (Table 1 and 2), the inhibition zone diameters were 30, 25 mm and 29, 20 mm at the higher and the lower concentrations, respectively. The result agreed with that reported by Chung *et al*. (1993) who stated that *Salmonella typhimurium* was sensitive to tannic acid, with antimicrobial activity at 10 to 20 g L^{-1} of tannic acid.

Tannic acid and natural tannins also showed antibacterial activity of 29-25 mm and 20-15 mm, respectively, against *Escherichia coli* and this result agreed with that of Irobi *et al*. (1994),

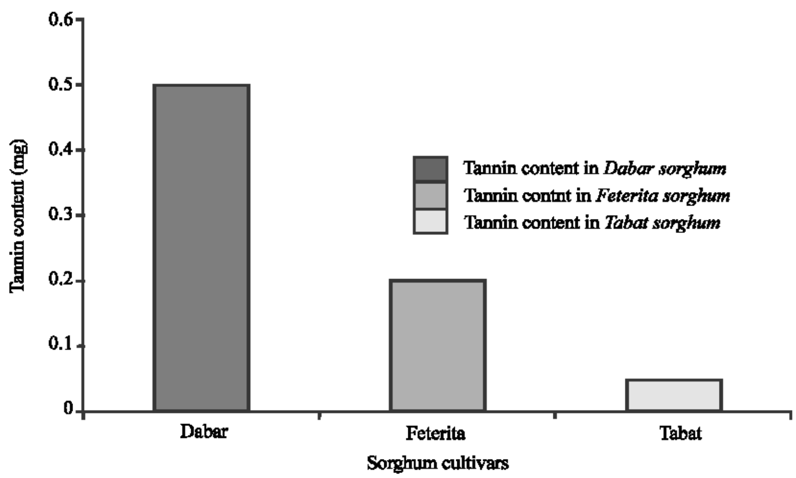

Fig. 1: Level of Tannin content in sorghum cultivars

Table 1: The antibacterial activity of tannic acid against examined organisms

Inhibition used	Solvent system	Concentration used (g L^{-1})	Inhibition diameter (mm)*		
			S.a	E.c.	Sa.t
Tannic acid	Distilled water	20	25	29	30
		10	20	25	25

*Examined organisms, S.a = *Staphylococcus aureus*, E.c. = *Escherichiac coli* Sa.t = *Salmonella typhymurium*

Table 2: The antibacterial activity of extracted tannins from sorghum grains against the examined organisms

Part extract used	Solvent system	Concentration used (mL)	Inhibition zone diameter (mm)*		
			S.a	E.c.	Sa.t
Seeds	Methanol	1:10	30	20	29
		1:10	15	15	20

*Tested organisms, S.a = *Staphylococcus aureus*, E.c. = *Escherichiac coli*, Sa.t = *Salmonella typhymurium*

Table 3: The antifungal activity of natural tannins

			Inhibition zone diameter (mm)*		
Part extract used	Solvent system	Concentration used (mL)	As.n	As.f	Sa.c
Seeds	Methanol	1:10	Complete growth	Complete growth	30
		1:10	Complete growth	Complete growth	25

*Tested organisms, As.n = *Aspergillus niger*, As.f. = *Aspergillus flavous*, Sa.c = *Saccharomyces cerivisae*

Table 4: The antifungal activity of tannic acid against examined organisms (moulds and yeast)

			Inhibition zone diameter (mm)*		
Part extract used	Solvent system	Concentration used (g L^{-1})	As.n	As.f	Sa.c
Tannic acid	Distilled water	20	Complete growth	Complete growth	25
		10	Complete growth	Complete growth	20

*Tested organisms, As.n = *Aspergillus niger*, As.f. = *Aspergillus flaveous*, Sa.c = *Saccharomyces cerivisae*

Hara and Ishigama (1989), who observed inhibition of *E. coli* by extracted tannins from plants. Also Chung *et al*. (1993) demonstrated that tannic acid and propyl gallate inhibited the growth of food borne bacteria, including *E. coli* and *Salmonella enteritits*.

Tannic acid and natural tannins showed a higher antibacterial activity against *Staphylococcus aureus*, the inhibition zone diameters were 25, 30 mm and 20, 15 mm at the higher and the lower concentrations, respectively. Those results agreed with that reported by Hada *et al*. (1989) and Sakanaka *et al*. (1989). They stated that *Staphylococcus aureus* were inhibited by condensed tannins and the growth of various diarrhea-causing pathogens was inhibited by tea extracts.

Antifungal Activity of Tannic Acid and Natural Tannins from Sorghum

The study indicated that tannic acid and natural tannins caused complete growth inhibition of *Aspergillus niger* and *Aspergillus flavous* (Table 3 and 4). Hitokoto *et al*. (1980) studied the effect of 13 herbal drugs and 7 commercial dry condiments on growth and toxin production by several toxigenic *Apergillus* species and found that powdered cinnamon was the most effective inhibitor. Hitokoto *et al*. (1980) reported that thyme caused 10-90% inhibition of the growth of three toxingenic *Aspergilli*, but showed nearly complete inhibition (86-100%) of their toxin production.

The data also indicated that tannic acid and natural tannins caused inhibition growth of *Saccharomyces cerivisae* type of yeast, when the inhibition zones were 25, 30 mm and 20, 25 mm at the higher and the lower concentrations, respectively. The result agreed to that reported by Mullins and Nesmith (1988) who reported that *Saccharomyces cerevisiae* was inhibited by sorghum grains by its tannins. Other authors demonstrated that tannic acid inhibited the growth of various yeast species some species were inhibited by tannins at 25 g L^{-1}, while others were inhibited at much higher concentration of tannins (125 g L^{-1}).

CONCLUSION

The objective of this research was to determine tannin content in sorghum cultivars, variation in the amount of tannin in different sorghum cultivars was found. The cultivar *Tabat* had the lowest tannin content than *Feterita* and *Dabar* samples. The microbiological analysis indicated that commercial tannins have high antimicrobial activity against many harmful microorganisms, especially *Salmonella typhimurium*. However, the inhibitory effect increased with an increase in inhibitor concentration.

More studies are needed to determine the antimicrobial activity of natural tannins from sorghum grains. Economic studies are needed to assess the extraction of tannin from sorghum grains and also the usage of sorghum tannins in food processing.

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