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Short Communication

Activity of essential oil and phenolic acid extracts of pepperfruit (Dennetia tripetala G. Barker; Anonaceae) against some food-borne microorganisms

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A previous report showed that the essential oil and phenolic acid extracts of pepperfruit (Dennetia tripetala) inhibited the growth of tomato-rot fungi. The study was subsequently extended to other food-borne microorganisms (Staphylococcus aureus, Salmonella sp. Pseudomonas aeruginosa., Proteus sp., Escherichia coli., Enterococcus faecalis., Serratia sp., Bacillus sp., Clostridium sp., Penicillium sp., Aspergillus flavus) isolated from food products. All the isolates were susceptible to the extracts with a minimum inhibitory concentration (MIC) range of 1.0-4.0 mg/ml. The essential oil inhibited the food-borne organisms better (MIC: 1.0-2.5) than the phenolic acid (MIC: 1.5-4.0). The challenge organisms in fresh, boiled or roasted beef, treated with the extracts were either not detected, declined significantly in number ($p<0.05$) or did not change significantly in population ($p>0.05$) after 7 days. A role for pepperfruit extracts in natural food protection is further indicated.

Key words: Essential oil, pepperfruit, dennetia tripetala, phenolic acid, food-borne microorganisms.

INTRODUCTION

Pepperfruit (Dennetia tripetala, G. Barker; Anonaceae) is an abundant edible fruit widely consumed in Southern Nigeria. It has been reported that the essential oil of the fruit contains nearly 80% -phenylnitroethane and is toxic to some insects (Agbakwuru et al., 1978; Iwuala et al., 1981). Ejechi et al. (1999) reported that the essential oil and phenolic acid extracts of the fruit inhibited the growth of tomato-rot fungi. The report concluded that the concern for carcinogenic potentials of synthetic preservatives may make pepperfruit attractive to food processors as a natural preservative if investigations show that it can inhibit other food-borne microorganisms. The report presented here is an extension of the work of Ejechi et al. (1999) to focus on the inhibition of other food-borne microorganisms by the essential oil and phenolic acid extracts of pepperfruit.

MATERIALS AND METHODS

Source and isolation of food-borne microorganisms

Unprocessed beef, eggs, okro fruit, bread, biscuits, groundnut and orange juice were purchased from the open market in the University town of Abraka, Nigeria and transferred to the laboratory for the isolation of the microorganisms (Staphylococcus aureus, Salmonella sp., Pseudomonas aeruginosa, Escherichia coli, Enterococcus faecalis, Serratia sp., Proteus sp., Bacillus sp., Clostridium sp., Penicillium sp., Aspergillus flavus) used for the experiment. The standard isolation protocol that includes blending in sterile normal saline and suitable serial dilution, before plating on suitable media (trypticase soy agar, de-oxycholate, citrate agar, bismuth sulphite medium, glucose azide agar, blood agar, MacConkey agar and potato dextrose agar). The identity of the bacterial isolates was confirmed with standard biochemical tests (e.g. Gram-stain, spore stain, starch and urea hydrolysis, coagulase, lactose fermentation, motility, swarming movement etc) and pigmentation (Buchanan and Gibbons, 1974; Starr et al., 1981). The taxonomic keys of Ainsworth et al.(1973) was used in the determination of the identity of the fungal isolates.

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Extraction of essential oil and phenolic acid

The steam distillation method (Durst and Gokel, 1987) was used for the extraction of essential oil from the pepperfruit while the phenolic acids was extracted by the procedure of McMurrough (1992) as described by Ejechi et al. (1999).

Susceptibility test

Molten nutrient agar (Oxoid) was incorporated with 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0…10.0 mg phenolic or essential oil extract/ml. Thereafter 3 replicate plates per isolate were inoculated by spread plate method using 0.1 ml normal saline suspension of 105 bacterial cells or fungal spores. The lowest concentration that failed to give any visible growth after incubation (24 - 48 h) at room temperature (30±20C) was regarded as the minimum inhibitory concentration (MIC).

Challenge test

Fresh beef obtained from a slaughterhouse under aseptic conditions was cut to 1 g pieces and rapidly immersed in ethanolic solution of pepperfruit essential oil or phenolic acid for 5 min. Thereafter they were placed in a dessicator at 400C for 15 min to allow the alcohol, evaporate. The uptake of the essential oil or phenolic acid by the beef was determined by weight difference. Unimmersed beef served as control because the beef was not dried to constant weight since it will defeat the objective of using fresh beef. The immersion in the extract solutions was repeated where necessary till an uptake of 3.0 mg/g beef was attained. The pieces of beef were subsequently rapidly transferred to sterile 150 ml flasks and inoculated with sterile tap water suspension of 105 bacterial cells or fungal spores. Three replicate flasks were used for each test organism per phenolic acid or essential oil extract. The control beef was inoculated but not treated with extract. The flasks were set -aside on the laboratory bench at room temperature (30±20C) for 7 days before they were analyzed for microbial population by plate count using nutrient agar. The above procedure was repeated with boiled and oven-roasted beef.

Table 1. Susceptibility of food-borne microorganisms to pepperfruit extracts.

Organism	Minimum inhibitory concentration (mg/ml)	
	Essential oil	Phenolic acids
Staphylococcus aureus	1.0	1.5
Salmonella sp.	0.5	1.0
Pseudomonas aeruginosa	2.5	3.5
Proteus sp.	0.5	1.5
Bacillus sp.	2.5	3.0
Escherichia coli	1.0	2.0
Serratia sp.	0.5	1.0
Enterococcus faecalis	1.0	2.0
Clostridium sp.	2.0	3.0
Penicillium sp.	2.5	3.5
Aspergillus flavus	2.5	3.0

RESULTS AND DISCUSSION

All the test organisms were susceptible to the phenolic acid and essential oil extracts although the MIC varied and susceptibility was more with the essential oil (Table 1). The greater antimicrobial activity of essential oil was also observed in a previous report on tomato-rot fungi (Ejechi et al., 1999). Extracts of higher plants contain a variety of phenolics and essential oil that are inhibitory to microorganisms (Stafford, 1974; Nakatani, 1994) and the antimicrobial activities of phenolics were indicated in plant disease resistance (Matern and Kneusel, 1998). Capsaicin or capsaicinoid compounds usually associated with red pepper possess antimicrobial activity (Nakatani, 1994) and may be present in the extract because of the pungent hot taste and lachrymation experienced when pepperfruit is consumed. The essential oil contain nearly 80% -phenylnitroethane and has been shown to be insecticidal (Agbakwuru et al., 1978; Iwuala et al., 1981) and inhibitory to tomato-rot fungi (Ejechi et al., 1999).

Table 2 presents the results of the challenge tests with regards to the protection of the test pieces of beef with essential oil. With the exception of P. aeruginosa and Bacillus sp., and the two test fungi all the organisms were not detected after 7 days. The population of those that were detected declined significantly after 7 days. The inference is that the antimicrobial activity of the pepperfruit essential oil is not limited to in vitro observation despite the failure to eliminate P. aeruginosa, Bacillus sp., Penicillium sp. and A. flavus. It should be noted that P aeruginosa is well known to be a versatile organism capable of survival in many hostile surroundings. On the other hand, the Bacillus sp. and the two fungi may have shown greater tolerance because of their spores. This observation is supported by the comparatively higher MIC values of the test organisms, (P aeruginosa, Bacillus sp. Penicillium sp. and A. flavus). With regards to beef protection by the phenolic acid, a static effect was observed (Table 3). The population of the challenge organisms either remained the same with the inoculum size or the changes observed in the population of some of the organisms were not significant (Table3). This not a surprise because it was observed earlier that the phenolic acid was less active than the essential oil as indicated by the MIC values.

The results of this study shows that extracts of pepperfruit can play a significant role in food preservation and protection against pathogens. Particularly interesting is that it could retard microbial growth in fresh beef for the 7 days it was observed. This may have potential applications in the distribution of fresh meat to rural areas of developing countries where refrigeration is not available due to the absence of electricity supplies. A major hindrance that would need to be overcome is the problem of imparting colour to the meat which consumers may find objectionable and the availability of pepperfruit. A combination of low concentration of pepperfruit extract

Table 2. Growth of challenge organisms in beef treated with essential oil extract of pepperfruit.

Challenge organisms	Mean microbial population (cfu/g) 7 days after treatment		
	Fresh beef	Boiled beef	Roasted beef
S. aureus.	ND	ND	ND
Salmonella sp.	ND	ND	ND
P. aeruginosa	2.0×10^2	1.5×10^2	0.4×10^2
Proteus sp.	ND	ND	ND
Bacillus sp.	1.2×10^2	1.8×10^2	0.6×10^2
E. coli	ND	ND	ND
Serratia sp.	ND	ND	ND
E. faecalis	ND	ND	ND
Penicillium sp	7.5×10^2	2.5×10^3	3.0×10^3
A. flavus	6.8×10^2	8.5×10^2	8.8×10^3

Values in the table are significantly different (t-test; $P<0.05$) from inoculum size (10^5 cfu/g); ND, not detected.

Table 3. Growth of challenge organisms in beef treated with phenolic acid extract of pepperfriut.

Challenge organisms	Mean microbial population (cfu/g) 7 days after treatment		
	Fresh beef	Boiled beef	Roasted beef
S. aureus.	1.20	1.10	0.90
Salmonella sp.	0.88	0.92	0.90
P. aeruginosa	1.30	1.30	1.00
Proteus sp.	0.85	0.86	0.85
Bacillus sp.	1.00	1.00	1.00
E. coli	1.20	1.00	0.95
E. faecalis	1.00	1.00	1.00
Serratia sp	0.96	0.94	0.90
Penicillium sp	1.00	1.00	1.30
A. flavus	0.95	0.92	1.20

Values in the table are not significantly different (*t*-test; $P>0.05$) from inoculum size (10^5 cfu/g).

and another mild preservative may solve the problem of colour in accordance with the concept of hurdle technology (Leistner and Gould, 2002) as an on-going investigation tends to suggest. With processed food such as roasted meat, the colour need not be a problem. Besides the lachrymation effect of the pepperfruit in roasted meat is desirable to Nigerians who generally relish pepper in food. However, pepperfruit trees have been steadily declining in Nigeria in the past 10 years due to deforestation. The manifestation of this is the skyrocketing cost of pepperfruit. Perhaps when industrialists begin to realize the potentials of the fruit in the food industry the trees would be replanted. We hope that this contribution will provide the impetus.

REFERENCES

Agbakwuru EOP, Osisiogu IUW, Uguchgukwu EN (1978). Insecticides of Nigerian vegetable origin II: Some nitroalkanes as protectants of stored cowpeas and maize against insect pests. Nigr. J. Sci.12:493-504.

Ainsworth GC, Sparrow FK, Sussman AS (1973). The Fungi Vol. 1VA: A Taxonomic Review with keys; Ascomycetes and Fungi imperfectii, London: Academic Press.

Buchanan RE, Gibbons NE (1974). Bergey's Manual of Determinative Bacteriology 8th edition, Baltimore: Williams and Wilkins.

Durst HD, Gokel GW (1987). Experimental Organic Chem. pp. 113-117. New York: McGraw Hill Book Company.

Ejechi BO, Nwafor OE, Okoko FJ (1999). Growth inhibition of tomato-rot fungi by phenolic acids and essential oil extracts of pepperfruit (Dennetia tripetala) Food Res. Intl. 32:395-399.

Iwuala MOE, Osisiogu IUW, Agbakwuru EOP (1981). Dennetia oil, a potential new insecticide: Tests with adults and nymphs of Periplanata Americana and Zonozerus variegates. J. Economic Entomol. 74:249-252.

Leistner L, Gould G (2002). Hurdle Technologies: Combination Treatments for Food Stability, Safety and Quality Lond. Kluwer Academic/Plenum Publishers.

Matern U, Kneusel RE (1998). Phenolic compounds in plant disease resistance. Phytoparasitica. 16:153-170.

McMurrough I (1992). Analysis of the Brewing Industry In MR Smyth (editor), Chemical Analysis in Complex Materials (pp. 88-148). England: Ellis Horwood Limited.

Nakatani N (1994). Antioxidative and antimicrobial constituents of herbs and spices. In G. Charalambus (editor), Spices, herbs and edible fungi (pp.251-271). Amsterdam: Elsevier.
Starford HA (1994). Activation of Hydroxy-cinnamate hydrolase in extracts of Sorghum. Plant Physiol. 54:686-689.
Starr MP, Stolp H, Trupe HG, Below AP, Shlegel HG (1981). The Prokaryotes. A hand book of Habitats , Isolation and Identification of Bacteria, Vols. I and II, Berlin: Sringer-Verlag.