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Growth inhibition of tomato-rot fungi by phenolic acids and essential oil extracts of pepperfruit (*Dennetia tripetala*)

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Abstract

Phenolic and essential oil extracts of pepperfruit (*Dennetia tripetala*) showed antifungal activity against *Saccharomyces cerevisiae*, *Saccharomyces* sp., *Candida tropicalis*, *Candida* sp., *Cryptococcus* sp., *Geotrichum* sp., *Rhizopus stolonifer*, *Aspergillus niger* and *Fusarium* sp. isolated from spoilt tomato fruits and cultured on agar plates. Antifungal activity of phenolic and essential oil extracts was observed at inhibitory concentrations (IC) in the range of 2.5–6.5 and 1.5–3.0 mg/ml tomato/glucose medium respectively. Combination of phenolic and essential oil extracts at concentrations below IC values significantly retarded growth of fungi resident in blended freshly harvested and open market tomato fruits. These fungi were not detected in the tomato after 1 month storage when mild heat (80°C for 1 min) and NaCl (10 mg/g) were combined with the phenolic and essential oil extracts as preservative hurdles. The population of challenge fungi in blended tomato treated with the same low concentration of the extracts and salt but without heat hurdle, steadily declined till none was detected after 3 months. Being an edible fruit, pepperfruit extracts may prove a useful natural preservative in food processing.

Keywords: Tomato; fungi; Pepperfruit; Phenolics; Essential oil

1. Introduction

Tomato (*Lycopersion esculentum*) fruit is an abundant crop used by many in Nigeria and indeed all over the world for making stew and delicacies. The importance of tomato is further emphasised by recent reports (Clinton, 1998) that epidemiologic studies implicated lycopene, a major constituent of tomato, in the prevention of cardiovascular disease and cancer of the prostrate. It is however, a perishable crop, which is not abundantly available all year round in Nigeria because it is climatic and this is worsened by very limited storage facilities. The cost of energy and erratic power supply limits the use of low temperature storage although chilling susceptibility is another problem. Although conventionally preserved tomato sauce or puree in cans and bottles are on the market; they are not within reach of the low income group because of cost. Thus, there is the need for an alternative method of preserving tomatoes which often lay wasted in the open market and on roadsides during the period of abundance in Nigeria. For a developing country, preservation should be simple and inexpensive (Leistner & Gorris, 1995).

Many reports (e.g. Zaika, 1988; Beuchat & Golden, 1989; Irobi, 1992; Nakatani, 1994) show that spices, herbs and other plant material possess antimicrobial activity. One of the many tropical plants that may posses antimicrobial activity is pepperfruit (*Dennetia tripetala* G. Barker; Anonaceae). It is an abundant edible fruit widely consumed in the southern part of Nigeria. The essential oil of the fruit has been reported to be toxic to some insects (Agbakwuru et al., 1978; Iwuala et al., 1981). There is paucity of information on the antimicrobial activity of the extract of pepperfruit. The strong pungent taste of pepperfruit when eaten causes severe lachrymation which suggests the presence of capsaicin or capsaicinoids found in red pepper. Capsaicin possesses some antimicrobial activity (Nakatani, 1994).

In view of the abundance and edible nature of pepperfruit, it would be of economic advantage if any of its extracts can inhibit the growth of food deteriorating

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microorganisms. The report presented here is on the effect of phenolic and essential oil extracts of pepperfruit on the growth of tomato-rot fungi.

2. Materials and methods

2.1. Isolation of tomato-rot fungi

Yeasts and moulds were isolated from deteriorating tomato fruits purchased from the open market using potato dextrose agar (PDA) containing 100 μg chloramphenicol per ml. The yeasts were identified by the simplified identification method of Deak and Beuchat (1987) and Deak (1993) while the moulds were identified with the aid of the keys of Ainsworth et al., (1973) and Webster (1977). The yeasts and moulds were maintained on PDA slants at 4°C till needed.

2.2. Extraction of phenolic acids

The extraction of the phenolic acids was by the procedure of McMurrough (1992). Ripe (reddish) pepperfruits were obtained from the market, shade-dried at ambient temperature, and ball-milled. Thereafter, it was extracted with acetone/water (3:1) for 72 h at room temperature (30 ± 2°C) and filtered with Whatman No. 1 filter paper. The filtrate was saturated with NaCl to promote phase separation. The upper phase which contained the phenolics was recovered and concentrated *in vacuo* at 40°C using a rotary evaporator (Buchii type). The yield of the viscous reddish-brown residue was 7.6 g/100 g pepperfruit.

2.3. Extraction of essential oil

Steam distillation was used for the extraction of the essential oil (Durst & Gokel, 1987). Milled pepperfruit weighing 50 g was placed in a round bottom flask containing 150 ml water and distilled. The distillate was saturated with NaCl and transferred to a separatory funnel where it was extracted with diethyl ether. The organic phase was recovered and concentrated on a steam bath. The yield was 2.8 g/100 g pepperfruit.

2.4. Susceptibility test

The minimum inhibitory concentration (MIC) was determined on tomato sauce/glucose 2 agar (TGA). It was prepared by boiling 300 g (wet weight) tomato in 1000 ml tap water for 30 min and mixing the filtrate with glucose (10 g/l) and agar power (15 g/l) before autoclaving at 121°C for 15 min. The molten TGA was incorporated with 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0...10 mg phenolic or essential oil extract/ml. Three replicate plates per organism were inoculated by spread plate method using 0.1 ml water suspension of 10^4 yeast cells or spores of mould isolates. After incubation at room temperature for 24–48 h the lowest concentration that failed to give any visible growth was regarded as the MIC.

2.5. Effect of extracts on growth of fungi in blended tomato

Freshly harvested tomato fruits were collected directly from the farm in polyethylene bags previously soaked overnight in 75% alcohol. The fruits were blended and 5 g quantities were dispensed into sterile 250 ml flasks. The yeast suspension was prepared from sterile distilled water washings of 24 h PDA cultures serially diluted to obtain the desired population. This was checked by microscopic and plate counts. A similar procedure was used for the spores except that 5-day old PDA cultures were used to give time for adequate sporulation. Thereafter, the fungal population was determined on PDA/chloramphenicol plates before treatment with single or combined preservatives (hurdles) according to the following schedule:

Heat (80°C for 1 min); NaCl (10 mg/g tomato); Phenolic extract; essential oil; Heat/NaCl; Heat/phenolic; heat/essential oil; NaCl/phenolic; NaCl/essential oil; phenolic/essential oil; heat/NaCl/Phenolic; heat/NaCl/essential oil; NaCl/phenolic/Essential oil; heat/phenolic/essential oil; heat/NaCl/phenolic/essential oil.

Five replicate flasks were used for each of the treatment listed above. The concentrations of the pepperfruit extracts applied were below the MIC value for the most susceptible of the test fungi (Phenolic extract, 2 mg/g; essential oil, 1 or 1.3 mg/g tomato).

The cotton-wool-plugged flasks were set aside for one month on the laboratory bench at room temperature and subsequently analysed for fungal population. The procedure includes appropriate serial dilutions with tap water to minimise the carry-over effect of the preservatives. The entire procedure was repeated with tomato fruits purchased from the open market. The blended tomato in all cases were analysed before and after incubation for total carbohydrate using the Clegg-Anthrone method (Josyln, 1970).

2.6. Challenge test

In view of the possibility of re-contamination after processing when the heat hurdle will be absent, a challenge test using the extracts and NaCl as the preservative hurdles was performed. Flasks containing blended autoclave-sterilised tomato were incorporated with combined hurdles (NaCl, 10 mg/g; phenolic extract, 2 mg/g; essential oil, 1.3 mg/g) and inoculated with aqueous suspension of 10^4 *A. niger* spores or cells

of *Candida* sp. per g blended tomato. Initial tests indicated that *Candida* sp. and *A. niger* were the most resistant to the phenolic and essential oil extracts (highest MIC) hence they were used. Similarly, the combination of hurdles used was earlier found to inhibit tomato-rot fungi in unchallenged tests. The cotton-wool-plugged flasks were set aside as before for periods of 2, 4, 6, 8, 10 and 12 weeks before analysis for population changes of the test fungi.

Table 1
Susceptability of tomato-rot fungi to phenolic and essential oil extracts of pepperfruit

Fungi	Minimum inhibitory concentration (mg/mL)	
	Phenolic	Essential oil
Saccharomyces cerevisiae	2.5	1.5
Saccharomyces sp.	3.0	1.5
Candida tropicalis	5.5	2.5
Candida	6.5	3.0
Cryptococcus sp.	4.5	2.0
Geotricum sp.	4.5	1.5
Rhizopus stolonifer	3.5	1.5
Aspergillus niger	5.5	3.0
Fusarium sp.	5.0	2.5

3. Results

The fungi isolated from the tomato fruits (Table 1) are among the usual organisms associated with deterioration of fruits and vegetables (Deak, 1991). The isolates were susceptible to the pepperfruit extracts to varying degrees (Table 1).

Table 2 presents the results of an attempt at finding the most suitable combination of hurdles, using the lowest possible concentration of the pepperfruit extracts, to inhibit the resident tomato-rot fungi. The population of the resident fungi in freshly harvested and open market tomato was 0.7×10^2 and 2.1×10^2 colony forming units (CFU) per g tomato respectively, before treatment. The single hurdles failed to inhibit fungal growth when compared to counts before treatment (initial counts). On the other hand all double hurdles

Table 2
The effect of pepperfruit extracts, NaCl and mild heat treatment or their combinations on the growth of fungi resident in tomato at room temperature (30 ± 2°C)

Preservative treatment			Freshly harvested tomato			Market tomato	
Pepperfruit extract (mg/g)		Heat (80°C for 1 min)	NACl (10 mg/g)	Mean fungal[a] count ($\times10^2$ cfu/g) after 1 month $n=5$	Loss of carbohydrate (%) $n=5$	Mean fungal[a] count ($\times10^2$ cfu/g) after 1 month $n=5$	Loss of carbohydrate (%) $n=5$
Phenolic	Essential oil						
2.0	–	–	–	16.0[b]	31.2	260.0[b]	51.6
–	1.0	–	–	5.6[b]	21.7	140.0[b]	47.3
–	1.3	–	–	1.2[b]	15.8	120.0[b]	46.6
–	–	+	–	4.9[b]	18.9	12,0000.0[b]	86.9
–	–	–	+	300.0[b]	48.2	18,000.0[b]	88.6
2.0	1.0	–	–	0.3[c]	0.0	1.2[c]	0.9
2.0	1.3	–	–	0.2[c]	0.0	1.1[c]	0.8
2.0	–	+	–	0.5[c]	0.0	1.3[c]	1.4
2.0	–	–	+	0.8[d]	0.0	78.0[b]	41.4
–	1.0	+	–	0.3[c]	0.0	1.2[c]	1.0
–	1.0	–	+	0.7[d]	0.0	82.0[b]	43.5
–	1.3	+	–	0.3[c]	0.0	1.2[c]	0.9
–	1.3	–	+	0.7[d]	0.0	170.0[b]	59.7
2.0	1.0	+	–	0.0	0.0	<0.2[c]	0.0
2.0	1.0	–	+	<0.2[c]	0.0	0.3[c]	0.0
2.0	1.3	+	–	0.0	0.0	<0.2[c]	0.0
2.0	1.3	–	+	<0.2[c]	0.0	0.3[c]	0.0
2.0	–	+	+	<0.2[c]	0.0	0.9[c]	0.0
–	1.0	+	+	<0.2[c]	0.0	0.8[c]	0.0
–	1.3	+	+	<0.2[c]	0.0	0.7[c]	0.0
2.0	1.0	+	+	0.0	0.0	0.0	0.0
2.0	1.3	+	+	0.0	0.0	0.0	0.0
Control	–	–	–	$>10^{10}$[b]	99.2	$>10^{10}$[b]	88.9

[a] Initial fungal counts (before treatment) were, 0.7×10^2 and 2.1×10^2 for freshly harvested and market tomato respectively.
[b] significantly higher than initial count ($p<0.05$;
[c] significantly lower than initial count, ($p<0.05$); c not significantly different from initial count ($p<0.05$) comparison was by *t*-test.
[d] +, treated; –, not treated.

Table 3
Growth of Challenge fungi in blended tomato fruits treated with combination of salt (10 mg/g) and phenolic (2 mg/g) and essential oil (1.3 mg/g) extracts of pepperfruit

Storage (weeks)[a]	Mean fungal counts[b]($\times 10^2$ cfu/g)	
	Candida sp. $n=5$	*Aspergillus niger* $n=5$
0	100.0	100.0
2	50.0	89.0
4	1.5	20.0
6	0.4	6.0
8	0.0	1.8
10	0.0	0.3
12	0.0	0.0

[a] Storage was at room temperature (30 ± 2°C).
[b] Counts in untreated (control) tomato was; $>10^{10}$ cfu/g by the 12th week.

except where salt was involved significantly reduced fungal population. Table 2 also shows that a combination of three hurdles was better than the double hurdles. Indeed no fungus was detected in freshly harvested tomato treated with a phenolic/essential oil/heat combination. The various combinations of the hurdles prevented loss of carbohydrates in freshly harvested tomato while combination of three or four hurdles prevented loss in market tomato (Table 2).

With regard to challenge tests the fungal population steadily declined till they were not detected at the end of the 3 month storage period (Table 3). Table 3 also shows that populations of *Candida* sp. declined faster.

4. Discussion

The phenolic and essential oil extracts of pepperfruit showed antifungal activities. Extracts of higher plants are known to contain a variety of growth inhibitory phenolic and essential oil (Stafford, 1974; Nakatani, 1994). The antimicrobial activities of phenolics is further indicated by their role in plant disease resistance (Matern & Kneusel, 1988). Phenolics interfere with the integrity of cell membranes or inhibit the germination of spores (Russel & Chopra, 1990). Although the active ingredients in the pepperfruit phenolic extract is yet to be identified the pungent hot taste and lachrymation experienced when consumed, suggests the presence of capsaicin or capsaicinoid compounds found in red pepper. Capsaicin possesses some antimicrobial properties (Nakatani, 1994). With regards to the essential oil of pepperfruit, previous reports have shown that it contains nearly 80% β-phenylnitroethane which indicated insecticidal activities (Agbakwuru, 1978; Iwuala et al 1981). From the results here, it is plausible that the biocidal potential of the essential oil was extended to tomato-rot fungi.

The results suggest that the extracts alone can prevent the deterioration of tomato by fungi. This would entail the use of concentrations, which may affect the sensory properties of the tomato. For example preliminary tests showed that high concentration of phenolic extract tended to darken the blended tomato colour. It therefore, became necessary that other hurdles (e.g. heat and salt) be included in accordance with the concept of hurdle technology. The heat may have reduced the fungal population and perhaps sublethally injured the spores, while salt may have imposed additional stress due to lowered water activity. This may have culminated in the fungal deteriogens becoming more vulnerable to the pepperfruit extracts. This inference is supported by the observation that it was only the combination of the four hurdles that eliminated the fungi in the much more contaminated tomato after one month. Micro-organisms strain every repair mechanism to overcome hurdles and by doing this, may become metabolically exhausted and die. This is authosterilisation (Leistner & Gorris, 1995).

The combination of essential oil and phenolic extract probably had a fungistatic effect at the concentration used. The absence of any loss of carbohydrate in freshly harvested tomato and less than 1% loss in the market tomato (Table 2) when the fungi were not completely eliminated supports this deduction. This fungistatic action is important because of the possibility of recontamination when there will be no heat hurdle to cross. The results of the challenge tests (Table 3) indicate that this fungistatic action may have been enhanced when the two extracts were combined with salt. This hypothesis is supported by the steady decline in challenge fungal population till they were not detected after three months storage. This is consistent with the phenomenon of autosterilization.

It can be concluded from the results of this study that the extracts of pepperfruit may be useful and economically feasible in tomato processing. As to questions on possible toxicity, the fact that it is widely consumed in southern Nigeria is indicative of its non-toxicity to man. Besides Iwuala et al., (1981) reported that rabbits and guinea pigs showed no adverse effects when they were fed with essential oil as pellet impregnations. With the increasing consumer demand for naturally preserved food, pepperfruit may become attractive to food processors especially when further investigation show that it can control many food spoilage agents. This will be the subject of future study.

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References

Agbakwuru, E. O. P., Osisiogu, I. U. W., & Ugochukwu, E. N. (1978). Insecticides of Nigerian vegetable origin II. Some nitroalkanes as protectants of stored cowpeas and maize against insect pests. *Nigerian Journal of Science, 12*, 493–504.

Ainsworth, G. C., Sparrow, F. K., & Sussman A. S. (1973). *The Fungi Vol. IVA: A Taxonomic Review with Keys; Ascomycetes and Fungi Imperfection*. London: Academic Press.

Beuchat, L. R., & Golden, D. A. (1989). Antimicrobials occurring naturally in foods. *Food Technology, 43*, 134–142.

Clinton, S. K. (1998). Lycopene; chemistry, biology and implications for human health and diseases. *Nutrition Reviews, 56*(2), 35–51.

Deak, T., & Beuchat, L. R. (1987). Identification of food-borne yeasts. *Journal of Food Protection, 50*, 243–264.

Deak, T. (1991). Food-borne yeasts. *Advances in Applied Microbiology, 36*, 179–278.

Deak, T. (1993). Simplified techniques for identifying Food-borne yeasts. *International Journal of Food Microbiology, 19*, 15–26.

Durst, H. D., & Gokel, G. W. (1987). *Experimemtal Organic Chemistry*. pp.133–117. New York: McGraw Hill Book Company.

Irobi, O. N. (1992). Activeties of *Chromolaena odorata* (Compositae) leaf extract against *Pseudomonas aeruginosa* and *Streptococcus faecalis*. *Journal of Ethnopharmacology, 37*, 81–83.

Iwuala, M. O. E., Osisogu, I. U. W., & Agbakwuru, E. O. P. (1981). Dennetia oil, a potential new insecticide: Tests with adults and nymphs of *Periplaneta americana* and *Zonozerus variegatus*. *Journal of Economic Entomology, 74*, 249–252.

Joslyn, M. A. (1970). *Methods in Food Analysis* (2nd ed). New York: Academic Press.

Leistner, L., & Gorris, L. G. M. (1995). Food preservation by hurdle technology. *Trends in Food Science and Technology, 6*, 41–46.

Matern, U., & Kneusel, R. E. (1988). Phenolic compounds in plant disease resistance. *Phytoparasitica, 16*, 153–170.

McMurrough, I. (1992). Analysis in the brewing Industry. In M. R. Smyth, *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, *Spices, Herbs and Edible Fungi*, (pp. 251–271). Amsterdam: Elsevier.

Russel, A. D. and Chopra, I. (1990) *Understanding Antibacterial Action and Resistance* (pp. 95–131). New York: Ellis Horwood Limited.

Stafford, H. A. (1974). Activation of hydroxy—cinnamate hydrolase in extracts of Sorghum. *Plant Physiology, 54*, 686–689.

Webster. J. (1977). *An Introduction to Fungi* (2nd edn.). London: Cambridge University Press (pp. 105–273).

Zaika, L. L. (1988). Spices and herbs: their antimicrobial activity and its determination. *Journal of Food Safety, 9*, 97–118.