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Evaluation of Antimicrobial Properties of Some Medicinal Plants for Fresh Cassava Roots Preservation

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Abstract: The inhibitory effects of water, petroleum ether and ethanol extracts of three medicinal plants were investigated on the growth of *Botryodiplodia theobromae, Aspergillus flavus, Fusarium solani, Rhizopus sp., Mucor* and *Erwinia spp* isolated from rotted cassava roots on potato dextrose agar. The ethanol extracts of *garlic* and *Landolphia owerrience* gave a wider spectrum of activity against the pathogens compared to that of petroleum ether and water. The combination of the extracts of *Garlic* (*Alium sativum*) and *Garcinia kola* demonstrated a remarkable inhibition of the pathogens after 16 days in storage with 2% rot, while that of *Garlic* and *Landolphia owerrience* roots exhibited little or no activity unlike the individual extracts. The potency shown by the extracts in overcoming the colonies of the tested pathogens recommends their use as protectants rather than eradicants.

Key words: Medicinal plants, cassava root rot, pathogens, biological control

Introduction

Fresh cassava roots (*Manihot esculenta Crantz*), unlike those of most other roots or tuber crops, are highly perishable under ambient conditions. Primary and secondary deterioration are known to occur. Physiological changes begin within one day and it is enzymatically mediated with loss of root quality in just three days. Roots also become subject to fungal invasion, further exacerbating deterioration (Bertram, 1990). Postharvest decay of cassava is a complex matter involving a number of different species of fungi and bacteria. Primary deterioration is a physiological change characterized by an internal root discoloration called vascular streaking. The onset of primary deterioration and the rate at which it progresses, the intensity, pattern and distribution of the discoloration varies between cultivars and roots of the same plant. Some varieties deteriorate so fast that they become inedible 24 h after harvest (Booth *et al*., 1976), while others have been reported to show no sign of discoloration for 7 to 11 days at room temperature. Secondary deterioration is induced by micro-organisms that cause rotting.

Garlic, Landolphia owerrience roots and *Garcinia kola* possesses varying degrees of antimicrobial properties. *Garlic* is acclaimed to have wide range of medical applications. Okeke *et al*. (2001) reported that the extracts of *Landolphia owerrience* roots demonstrated a wide spectrum of antimicrobial activities while Iwu *et al*. (1999), reported that *Garcinia kola* has both antiparasitic and antimicrobial properties. It can also be used as a purgative. The objective of this study is to assess the antimicrobial properties of locally and readily available medicinal plants with a view to exploiting them for control of postharvest rot of cassava roots.

Materials and Methods

Sample collection: Wholesome and rotting cassava roots were harvested from NRCRI, Umudike cassava farms.

Isolation of the pathogens: Two freshly rotted pieces from surface sterilized cassava root were aseptically inoculated on potato dextrose agar (PDA) plates. It was replicated three times. They were incubated at room temperature (28°C). Observations were made daily for emergence of colonies for six days. Identification was done and compared with the illustrated genera of imperfect fungi by Barnette and Hunter (1972) and the class work of fungi by Dade and Gunnel (1969).

Establishment of pathogenicity: Thirty wholesome cassava roots were washed under running water and surface sterilized with 70% ethanol. Two groups of ten roots each were inoculated with the isolated organisms at concentration of 4.3×10^9 spores/mL at the rate of 10mL of suspension per root according to Okigbo and Ikediugwu, 2000. The remaining ten roots were spray-inoculated with the equivalent volume of blank sterile distilled water. The three groups were covered separately with sterile transparent polyethylene sheets and incubated on a raised platform at room temperature for five days. The cassava roots were examined on daily basis for the occurrence of rot and for the

determination of the root surface mycoflora according to Harley and Waid (1955).
Re-isolation of the inoculated pathogens was done and were subsequently identified and compared with the illustrated genera of imperfect fungi by Barnett and Hunter (1972).

Preparation of extracts: A 100g amount of the pulverized *Garcinia kola*, *Garlic* and *Landolphia owerrience* roots were continuously extracted with petroleum ether, water and ethanol in a soxhlet extractor for 2½ h. The solvents were subsequently distilled off in a rotatory evaporator. The extracts were poured into weighed flasks and further dried in a desiccating chamber to a constant weight. The dried extracts were exposed to ultra violet (UV) rays for 24 h. and checked for sterility by streaking on nutrient agar plates.

Determination of minimum inhibitory concentration (mic): The mic for the extracts were determined by two test methods: a modified agar-well diffusion method (Okeke *et al*., 2001) and a modified macro-broth dilution technique (Ibrahim *et al*., 1997). In the agar-well diffusion technique, a two-fold serial dilution of the extracts were prepared by first reconstituting in 20% dimethylsulphoxide (DMSO). They were diluted in sterile distilled water to achieve a decreasing concentration range of 50mgmL^{-1} to 8.021mgmL$^{-1.}$ A 100µL volume of each dilution was introduced in triplicate wells into potato dextrose agar (PDA) plates already seeded with the standardized inoculum (5×10^{5}) of the test fungal cells. The test plates were incubated at 28^{o}C for 72 h. The least concentration of each extract showing a clear zone of inhibition was taken as the mic.
In the macro-broth dilution technique, a two fold serial dilution of the reconstituted extracts were prepared in potato dextrose broth (PDB). Each dilution was seeded in triplicates with 100µL of the standardized suspension of the test fungal strain to achieve a final concentration of 5×105cfu/mL. The culture tubes were incubated at 28^{o}C for 72 h. The tubes were subsequently plated on solid PDA plates and incubated for 72 h at 28^{o}C. The least concentration showing zero growth on the PDA plates was taken as the mic.

Test for combined activity of the extracts: A total of three different combinations of the extracts were tested against the susceptible pathogens at a 50mgmL^{-1} concentration. The combinations were: *Landolphia owerrience* roots and *Garcinia kola*, *Landolphia owerrience* roots and *Garlic* and *Landolphia owerrience* roots, *Garcinia kola* and *Garlic*. The agar well diffusion method was used and the inhibition zone diameter measured to the nearest millimeter.

Treatment of cassava roots with plant extracts: Fresh and wholesome cassava roots were grouped into nine and replicated three times. The first three groups were sprayed separately with the individual extracts at a 50mgmL^{-1} concentration. The three different combinations of the extracts were also separately sprayed on the second three groups of cassava roots with the same concentration. They were inoculated with the four susceptible pathogens at a concentration of 4.3×10^{9} spores/mL at the rate of 10mL of suspension per cassava root according to Okigbo and Ikediugwu (2000). The remaining three groups were sprayed with the equivalent volume of blank sterile distilled water before spraying with the same concentration of the pathogens. The application method was also reversed by inoculating the pathogens first on the cassava roots before spraying with the extracts.

Results

Biodeterioration of cassava roots occurs in most cases after harvest. Micro-organisms isolated from the rotten cassava roots plated on potato dextrose agar (PDA) includes: *Botryodiplodia theobromae*, *Aspergillus flavus*, *Fusarium solani*, *Rhizopus sp*., *Mucor* and *Erwinia spp*. Pathogenicity test revealed that *B. theobromae*, *Rhizopus sp*., *Aspergillus flavus* and *Fusarium solani* were mostly implicated in cassava root rot under storage.
All the extracts reduced to a varying degree the radial growths of the pathogens (Table 1). The spectrum of activity against the pathogens by the extracts was in the following decreasing order: *Garlic*, *Landolphia owerrience* and *Garcinia kola*. The percentage rot ranged from 1% to 25% (Table 2). The combinations of *Garlic* and *Landolphia owerrience*, *Garcinia kola* and *Landolphia owerrience* exhibited little or no antimicrobial properties. However, the combination of *Garlic* and *Garcinia kola* gave the best result with only 2% rot after 16 days of storage.

Discussion

Deterioration of cassava roots is due to microbial attack, physiological changes and mechanical injuries. *B. theobromae*, *Rhizopus sp*, *Aspergillus flavus* and *Fusarium solani* were found to be the major causal organisms. Interestingly, the results obtained in the inhibition of the extracts against the postharvest pathogens of cassava roots, suggests that the extracts were bioactive (Table 1). The ethanol extracts of *Garlic* and *Landolphia owerrience* gave a wider spectrum of activity against the pathogens compared to that of petroleum ether and water. The effectiveness of ethanol extracts in inhibiting the biomass and the mycelial growth of the pathogens may be ascribed to the soluble nature of the antimicrobial properties of the test plant materials. Ebi and Ofoefule (1997) and Okeke *et al*. (2001) have reported the presence of the following phytochemical compounds in selected medicinal plants: glycoside, saponin, tannin, flavonoids, terpenoids and alkaloids.

Table 1: Percentage growth inhibition

	Ethanol extract (in days)				Water extract (in days)				Petroleum ether extract (in days)			
Extracts	6.0	8.0	12.0	14.0	6.0	8.0	12.0	14.0	6.0	8.0	12.0	14.0
Garlic	100.0	100.0	84.3	65.6	12.6	10.2	10.2	8.3	56.0	52.5	41.6	33.4
Landolphia owerrience	100.0	100.0	78.7	61.3	36.2	21.1	18.4	12.4	48.3	42.6	30.8	28.6
Garcinia kola	53.4	38.2	33.4	20.6	26.1	20.5	10.1	8.4	42.6	36.5	28.4	17.6
Control Ethanol	0.0	0.0	0.0	-	-	-	-	-	-	-	-	-
Water	-	-	-	-	0.0	0.0	0.0	0.0	-	-	-	-
Petroleum Ether	-	-	-	-	-	-	-	-	0.00	0.00	0.00	0.00

Note: Data are averages of three replications

Table 2: Incidence of rot during storage of cassava roots treated with Plant extracts

	% Rot during storage for a period of 6-16 days					
Treatment	6	8	10	12	14	16 (days)
Uninoculated (Control)	2.5	5	12	16	20	24
Ethanol	1	2	8	13	16	21
Petroleum ether	2	4	10	14	20	23
Water	3	5	13	17	21	25
Garlic	0	0	0	0	0	8
Landolphia owerrience	0	0	0	0	0	12
Garcinia kola	0	0	12	12	14	16
Garlic+Garcinia Kola	0	0	0	0	0	2
Garlic+Landolphia owerrience	2	5	8	15	18	20
Garcinia Kola+Landolphia owerrience	3	6	12	17	21	24

Note: Data are averages of three replications

Combination of Garlic and Garcinia Kola demonstrated a remarkable inhibition of the pathogens after the 16 days (2%) rot (Table 2). The combinations of *Garlic* and *Landolphia owerrience* roots, *Garcinia kola* and *Landolphia owerrience* roots exhibited little or no activity unlike the individual extracts. This could be as a result of the antagonistic properties of *Landolphia owerrience* roots. This finding is in line with the earlier report of Okeke *et al*. (2001). The contribution of the phytochemical properties of the extracts as a means of biocontrol is indicated not only by the *in vitro* inhibition of spore germination of all the tested pathogens, but also by the potency of the extracts against the colonies of the pathogens of the cassava roots.

The observed drastic suppression of the pathogens by the plant extracts suggest strongly that they are potent and are therefore suitable for postharvest control of cassava root rot. The efficiency shown by the extracts in overcoming the colonies of the tested pathogens recommends their use as protectants rather than eradicants. The overall strategy of bio-control fits in well with the current concerns of sustainable agriculture, whereby renewable resources are used, with less impact on the environment as a whole.

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