

Full Length Research Paper

Evaluation of two plant extracts for the control of post harvest fungal diseases of cassava (*Manihot esculenta* crantz) in Calabar, Nigeria

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Accepted 17, April 2014

The bioactive and efficacy of ethanolic extracts of *Zingiber officinale* Rosc. and *Gongronema latifolium* Benth. were investigated in vitro on causative agents of post harvest decay of cassava. The phytochemical screening of the two plants were obtained by analysis of their extracts using established procedures. Results revealed high presence of saponins, cyanogenetic glycosides and polyphenols in *Zingiber officinale*, as well as anthraquinones in *Gongronema latifolium*, with moderate presence of flavonoids, alkaloids and cyanogenetic glycosides in the two plants, indicating their antifungal potentials. The pathogens isolated were *Rhizopus stolonifer*, *Penicillium expansum*, *Fusarium moniliforme* and *Aspergillus niger*. The fungi were cultured with the two plant extracts at concentrations of 100%, 80%, 60%, 40%, 20% and 10%, using potato dextrose agar (PDA). The antifungal activity increased with increase in concentration of the plant extracts. At 100% concentration, extract of *Zingiber officinale* showed the highest inhibition of mycelia growth in *Aspergillus niger* (97.37±1.56%) while that of *Gongronema latifolium* was 88.60±0.85%. Specifically, extracts of *Zingiber officinale* was more effective in inhibiting growth of the fungal isolates; and *Aspergillus niger* was found to be most sensitive to the extracts.

Key words: Evaluation, plant extracts, control, postharvest, fungal diseases, cassava.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is a shrubby, short lived perennial plant with storage underground roots (tubers) which are edible, of the family Euphorbiaceae (Kochhar, 1986). The crop contains over 30% of starch and vitamin C, and calcium in its roots; while its leaves contain a high level of vitamin A and up to 17% protein (cassava. www.nutritiondata.com). The leaves are therefore, used as green vegetables (AIC, 2002). In West Africa and particularly Nigeria, cassava is eaten by all classes of people and is used in preparing garri (a flour-like meal), 'Akpu' (fermented cassava) and 'tapioca' (boiled cassava chips); while its peelings after elimination of the poisonous hydrocyanic acid (HCN) is used as fodder (Onwueme, 1978).

The main diseases affecting cassava in the field and

their causal agents are African cassava mosaic disease (ACMD) caused by African Cassava mosaic virus (ACMV), cassava bacterial blight (*Xanthomonas campestris* pv. *manihotis*), cassava brown leaf spot (*Cercosporidium henningsii*), cassava brown streak virus disease (potyvirus), cassava anthracnose (*Glomerella manihotis*) and cassava root rot (*Aspergillus niger*) (CAB International, 2005). The major problem of cassava in storage is the attack of storage pest (beetles and moths) like the larger grain borer (*Prosterphanus truncatus*) and the lesser grain borer (*Rhizopertha domestica*) which are serious pests of dried cassava and cereal grains (Stoll, 1996; James et al., 2000). Cassava unlike yam does not store well when harvested as it rapidly deteriorates due to invasion by pests and microbial agents like *Rhizopus* sp. and *Aspergillus* sp. (IITA, 1996); and therefore, must be peeled, chopped into chips and dried or processed into flour before storage to avoid rot (Cassava. www.foa.org).

Post-harvest storage of cassava in East Africa, infected with storage fungi was reported to have caused loss of

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nutrients, discoloration of the crop, mouldy smell and tasted, as well as production of mycotoxins (Natural Resources Int. Ltd, 2004). INPhO. cassava.www.foa.org). Mycotoxins in cassava, like in other grains, are highly poisonous, causing liver cancer; and the best-known mycotoxins are aflatoxins (caused by *Aspergillus flavus*), ochratoxins, patulin, and citrin. Mycotoxins are very stable once infected with cassava and other storage grains; and cannot be destroyed by boiling, pressing and processing (Elwell and Maas, 1995). Stored cassava products contaminated with mycotoxins are often destroyed to avoid aflatoxin infection; and so the only way of avoiding mycotoxins is to prevent fungal growth in cassava and other stored produce (Gwinner et. al, 1990). To prevent fungi contamination in stored cassava, the crop must be properly dried to 12-13% moisture, and any source of moisture should be avoided in the store (Elwell and Maas, 1995; The organic Farmer Magazine, 2007). In Kenya, the larger grain borer and other grain pests were effectively repelled by storing cassava in a fairly large amounts of dried lantana, eucalyptus or neem leaves (RRI/MAFF, 2004; Gwinner et.al., 1990; Neuenschwander, 2003).

Plant extracts have been recorded and shown to have significant activities against rot-causing organisms in fruits and other plant products (Awuah, 1989; Bankole and Adebajo, 1995 and Uzuegbu and Okoro, 1999). Also, the use of extracts from higher plants in the control of a wide range of fungal diseases that infect crop plants have been reported by previous researchers (Gerard-Ezhilan et al, 1994; Kurucheve et al, 1997 and Tiwari, 1997). Such bioactive compounds were used in preference to conventional fungicides as they are cheap, biodegradable, selective in their toxicity, eco-friendly and non phytotoxic (Singh et al., 1983; Singh and Dwivedi, 1997; Srivastava and Lal, 1997; Dubey, 1991 and Akueshi, et al, 2012).

Gongronema latifolium Benth is a weak, perennial green climbing plant of the family, Asclepiadaceae. The stem is soft and fibrous, and both the stem and leafy stalk produce milky latex when cut. The leaves are used for the treatment of diabetes and relieve of stomach ache (Okujagu, 2008), and other diseases in ethnomedicine. Okeke and Elekwa (2006) reported that leaf extracts of *G. latifolium* successfully lowered the blood glucose levels of alloxan-induced diabetic rats after 2 hours of administration. Alobi et al. (2012) while conducting a phytochemical screening of leaves of *G. latifolium* recorded high presence of anthraquinones, polyphenols, cyanogenic glycosides and saponins, thus, confirming the therapeutic potentials of this herb in ethnomedicine. However, literature on the use of this plant in the control of crop diseases is lacking or scarce.

Zingiber officinale Rosc, locally called ginger is an erect, perennial herb which consists of an underground stem (rhizome) of the family Zingiberaceae (Leung and Foster, 1996). It is a popular spice and contains a volatile

aromatic oil called gingerol (Gruenwald and Montavale, 1998); and a non volatile aromatic oil with a pungent taste called gingerin (Kochhar, 1986). The presence of these chemicals and other bioactive ingredients like phenols makes this plant useful in ethnomedicine, in the treatment of human diseases like cough, cardiovascular disorders, arthritic conditions, body inflammation and platelet aggregation (Fischer-Rasmussen et al., 1991). *Zingiber officinale* was also reported to significantly controlled fungal rot pathogens of yam (Okigbo and Nmeko, 2005); and also in the control of microorganisms causing deterioration of yam chips over *V. amygdalina* in vitro (Okigbo et al., 2012). Also, application of extracts of *Gmelina aborea* and *Zingiber officinale* significantly inhibited the mycelia growth and sporulation of *Colletotrichum capcici* (in vitro) the causal organism of brown blotch disease of cow pea (Ajayi and Olufolaji, 2008). Similar studies by Oluma and Garba (2004) also reported that extracts of *Eucalyptus globulus* and *Ocimum gratissimum* significantly reduced radial (mycelia) growth of *Pythium aphanidermatum* by 44.5-100%, with *E. globulus* being more potent. Also, radial growth and dry weight of the rice pathogens, *Helminthosporium oryzae* and *Rhizoctonia solani* were drastically reduced by the volatile oils from *Eucalyptus citridora* and its major constituent, citronella (Ramezani et al., 2002).

This study, therefore, investigates the phytochemical and evaluation of extracts of *Gongronema latifolium* and *Zingiber officinale* for the control of post harvest fungal diseases of cassava in Calabar, Nigeria.

MATERIALS AND METHODS

Plant materials

Fresh leaves of *Gongronema latifolium*, rhizome of *Zingiber officinale* and tubers of cassava used for this study were purchased from Marian Market in Calabar Metropolis. The plants were identified at the Herbarium unit of the Department of Biological Sciences, Cross River University of Technology, Calabar.

Isolation of Test Fungi from Cassava.

Partially decay tubers of cassava (200g) were washed in sterile distilled water in the laboratory at room temperature (27°C) for 4 days. Each petridish contained 4 pieces (2x2cm) of cassava. The partially decayed cassavawere soaked in a 500ml beaker containing sterile distilled water for 10 minutes. The contents of the beaker was stirred with a sterile glass rod to dislodge more microbial agents into the water. The cassava were later removed from the beaker and the contents filtered through sterile cheese cloth. Exactly 1.0ml aliquot of the

filtrate was aseptically inoculated into each of the 4 petridishes to obtain pure cultures and used as fungal inoculum for the plants. Observed fungal colonies were identified on the basis of spore characteristics and nature of mycelia using a compound microscope and identification guides.

Preparation of the Plant Extracts

Leaves of *Gongronema latifolium* and fresh rhizome of *Zingiber officinale* were thoroughly washed with sterile distilled water and the ginger was further chopped into smaller pieces; and both plants were dried in an oven at 45°C for 30 minutes. Both plants were pulverized separately with a household blender (model 830L, Hong Kong). The crude extracts of the two plant samples were prepared using standard procedures (Fato Pe *et al*; 1999; Mukhtar and Huda, 2005). This involved soaking 50g of the powdered extract in 95% ethanol for 48 hours at room temperature to allow for maximum extraction of the components. This was followed by evaporation of the filtrate to eliminate the solvent using a rotary evaporator (STUARC SCIENTIFIC, ENGLAND). The residue left was used as crude extract for each of the test plants, and stored in reagent bottles in the refrigerators at 12°C until needed.

Phytochemical screening

The phytochemical screening (qualitative determination) of the bioactive ingredients of the plants were determined using the methods of (Culier, 1982; Sofowora, 1984 and Gundiza, 1985).

Preparation of Extract Concentration

The undiluted extracts in the stock bottles were used as 100 percent concentration of the extracts. Also, 80ml of the 100 percent concentration of the extract was diluted with 20ml of sterile distilled water to obtain 80 percent concentration. The serial dilution procedure was continued to obtain lower concentrations of the extracts as follows: 100, 80, 60, 40, 20 and 10 percent.

Effect of Plant Extract

Five milliliter (5ml) from each of the concentration of the extract (10, 20, 40, 60, 80, and 100%) was dispensed into 9cm diameter petridish and agitated thoroughly with 20ml of melted PDA forming potato dextrose leaf extract agar (PDLA). The agar extract mixture was allowed to solidify and then inoculated centrally with a 5mm diameter mycelia disc obtained from the colony of 4-day

old culture of each of the test fungi using a sterile inoculating needle. PDA plates inoculated with the test fungi but without the extract served as the control. All the plants were incubated at 27°C, after which the zones of inhibition on colony diameter were measured for five days. Percentage inhibition was determined according to Whipps (1987).

$$\text{Percentage inhibition} = \frac{R_1 - R_2}{R_2} \times \frac{100}{1}$$

Where R_1 = furthest radial distance of fungus in treatment plates (PDA only).
 R_2 = furthest radial distance of fungus in treatment plates.

The experiment was repeated thrice in a randomized complete block design with 6 treatments and 3 replicates. Statistical Analysis Results were expressed as mean \pm SD. Comparison of mean of 3 determinations; and results adjudged significant at $P < 0.05$.

RESULTS

Results of the phytochemical screening of the ethanolic extracts of the test plants are summarized in Table 1. *Zingiber officinale* shows high presence of saponins, cyanogenetic glycosides and polyphenols with moderate presence of flavonoids and alkaloids. However, there was low presence of steroids while tannins and anthraquinones were absent or undetected. On the other hand, high levels of polyphenols and anthraquinones were recorded in *Gongronema latifolium* with moderate levels of flavonoids and cyanogenetic glycosides. Also, saponins and tannins showed low presence, while alkaloids and steroids were absent or not detected.

The microbial agents isolated from post harvest deterioration of cassava were: *Rhizopus stolonifer*, *Penicillium expansum*, *Fusarium moniliforme* and *Aspergillus niger*. The fungi and their prevalence of occurrence are shown in Table 2. The most prevalent fungus was *Rhizopus stolonifer* (40%), closely followed by *Aspergillus niger* (30%) while *Fusarium moniliforme* (10%) was least.

The antifungal activity of the extracts on the test fungi is shown in Table 3. The extracts inhibited the growth of the test fungi at all the concentrations. The extracts showed increased antifungal activity with a corresponding increase in the concentration of the extracts studied. The highest percentage inhibition of mycelia growth of the fungi was observed at 100% extract concentration of *Z. officinale*,

Table 1. Phytochemicals present in ethanolic extract of plants used.

Bioactive	<i>Zingiber officinale</i>	<i>Gongronema latifolium</i>
Compound		
Saponins	+++	+
Tannins	-	+
Flavonoids	++	++
Alkaloids	++	-
Cyanogenic Glycosides	+++	++
Polyphenols	+++	+++
Anthraquinones	-	+++
Steroids	+	
Key		
+	=	low presence
++	=	moderate presence
+++	=	High presence
-	=	Not present

Table 2. Percentage occurrence of Pathogens isolated from postharvest decay of cassava.

Fungi isolates	% occurrence
<i>Rhizopus stolonifer</i>	40
<i>Penicillium expansum</i>	20
<i>Fusarium moniliforme</i>	10
<i>Aspergillus niger</i>	30

with *A. niger* (97.37±1.56%), *F. moniliforme* (94.77±0.96%), *P. expansum* (83.73±0.80%) and *R. stolonifer* (71.50±0.79%) Table 3. At 10% concentration, the lowest antifungal activity was recorded in *G. latifolium* extract with *R. stolonifer* (5.13±0.21%), *P. expansum* (8.70±0.85%), *F. moniliforme* (13.67±1.00%) and *A. niger* (20.67±0.71%). Extract of *Z. officinale* significantly ($P>0.05$) showed a higher antifungal activity than *G. latifolium* at all the concentrations. At 100% concentration, *A. niger* emerged the most susceptible fungus with the plant extracts, as it recorded the highest inhibition with *Z. officinale* (97.37±1.56%) and *G. latifolium* (88.60±0.85%) over other fungi. The control plates, without the extracts showed no inhibition of the fungus.

DISCUSSION

The pathogens associated with the post harvest deterioration of cassava in this study were *R. stolonifer*, *P. expansum*, *F. moniliforme* and *A. niger*. *R. stolonifer* showed the highest occurrence, closely followed by *A. niger* while *F. moniliforme* was the least.

This study has shown that *R. stolonifer*, *P. expansum*, *F. moniliforme* and *A. niger* associated with the post

harvest deterioration of cassava were inhibited by the ethanolic extracts of *Z. officinale* and *G. latifolium* at all the concentrations in vitro. These results also agree with the reports of other workers on the inhibitory action of plant products employed on the mycelia growth and spore germination of other pathogenic fungi (Bankole and Adebajo, 1995; Uziegbu and Okoro, 1999; Kuruchev *et al*, 1997 and Tiwari, 1997). This corroborates the report of other workers (Okigbo and Nmeko, 2005; Okigbo *et al*, 2012 and Ajayi and Olufolaji, 2008), that *Z. officinale* is among important plants, whose extracts are capable of checking the spread of many fungal diseases of food crops. Also, a comparative study of the chemical analysis of the leaves of *G. latifolium*, *Lasianthera africana* and *Hansia crinata* concluded that *G. latifolium* had the highest anthraquinones, polyphenols, cyanogenic glycosides and flavonoids than the rest, indicating highest medicinal potency than the others in ethnomedicine (Alobi, 2012). Studies on this plant was also reported to be capable of relieving stomach ache (Okujagu, 2008); and also, successfully lowered blood glucose levels in alloxan-induced diabetic rats, two hours after inoculation (Okeke and Elekwa, 2006).

Extract from *Z. officinale* appeared to possess the strongest antifungal property against the mycelia growth of all the test fungi over *G. latifolium* at same concentrations.

Table 3. Effect of different concentration of extracts of *Zingiber officinale* and *Gongronema latifolium* on the growth of fungal isolates of cassava (incubated at 27°C for 5 days after inoculation) *Zingiber officinale* (Percentage inhibition)

Extract conc.(%)	<i>Z. officinale</i> (Percentage inhibition)				<i>G. latifolium</i> (Percentage inhibition)			
	<i>R. stolonifer</i>	<i>P. expansum</i>	<i>F. moniliforme</i>	<i>A. niger</i>	<i>R. stolonifer</i>	<i>P. expansum</i>	<i>F. moniliforme</i>	<i>A. niger</i>
Control (0)	0	0	0	0	0	0	0	0
10	6.57±0.70	11.47±0.85	16.37±0.67	31.43±1.01	5.13±0.21	8.70±0.85	13.67±1.00	20.67±0.71
20	16.50±0.75	21.57±0.75	33.40±0.70	51.53±0.81	15.47±0.49	16.60±0.92	27.50±0.95	44.37±0.60
40	34.50±0.66	41.47±0.96	64.53±0.70	75.73±0.91	30.67±0.96	36.40±1.37	48.57±0.87	62.60±0.66
60	51.67±1.17	65.50±0.62	76.30±0.89	80.77±0.90	46.70±0.53	53.73±1.00	64.73±1.01	73.53±0.71
80	61.50±0.82	70.47±0.67	81.57±1.22	91.60±0.80	56.53±0.65	68.47±0.67	73.83±0.90	81.57±0.75
100	71.50±0.79	82.73±0.80	94.77±0.96	97.37±1.56	67.57±0.76	75.7±0.98	83.00±1.41	88.60±0.85

* Values are mean± standard deviation from three replication

The efficacy of the antifungal property of *Z. officinale* over other plant extracts was also reported in the control of some crop plant diseases (Okigbo and Nmeke, 2005; Ajayi and Olufolaji, 2008 and Okigbo *et al*, 2012).

The high antimicrobial property exhibited by *Z. officinale* over *G. latifolium* in this study could be attributed to the high chemical potency (saponins, cyanogenetic glycosides, alkaloids and steroids) of its extract than that of *G. latifolium* (Odebiyi and Sofowora, 1978). The high fungitoxic effect of *Z. officinale* observed in this study also confirmed earlier studies on the volatile oil of the extract, as was reported to contain gingerol and other trihalogenophenols (Gruen-wald and Montaville, 1998) which are active ingredients responsible for the inhibitory effect of several fungal pathogens of food crops (Sofowora, 1984; Awua, 1989; Oluma and Garba, 2004 and Ramezani *et al*, 2002). The presence of these bioactive substances gives credence to its traditional use, in the treatment of human diseases like cough, body inflammation and platelet aggregation (Fischer-Rasmussen *et al*, 1991). Although the plant extracts effectively inhibited the growth of the fungal pathogens in vitro, work is ongoing in the green house and our experimental farm to confirm the efficacy of their leaves and other plant parts to control storage pests of cassava products (cassava chips and flour and other fungal diseases in vivo).

The fungicidal attribute of the two plant extracts on the fungal isolates of post harvest decay of cassava (in vitro) recorded in this work, can further be developed pharmacologically for the control of post harvest rot of

cassava caused by pathogenic fungi and other microbial agents of other crop diseases.

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