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ANTIFUNGAL POTENCIES OF LEAF EXTRACTS OF *Glyphaea brevis* AND *Spondias mombin* ON FUNGI IMPLICATED IN DRY ROT OF POSTHARVEST YAM

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Abstract
The sensitivities of selected fungal pathogens implicated in dry rot of postharvest yam to leaf extracts of Glyphaea brevis and Spondias mombin were evaluated. The cup-plate agar method was used to estimate the sensitivities of the ethanol leaf extracts of Glyphaea brevis and Spondias mombin on Aspergillus niger, Fusarium oxysporum, and Penicillium oxalicum. All the cold ethanol leaf extracts showed potencies on all test pathogens, while hot ethanol extract of Spondias mombin showed no potency on Penicillium oxalicum. The lowest minimum inhibitory concentration obtained for Spondias mombin and Glyphaea brevis were 1 x 10^{-4}mg/mL of cold extract on Aspergillus niger, and 4.0 x 10^{-4} mg/mL of hot extract on Aspergillus niger, respectiviely. The lowest potencies were seen on Fusarium oxysporum for both leaf extracts. Phytochemical analysis of the leaves revealed the presence of saponins and glycosides in Glyphaea brevis; and saponins, tannins, alkaloids, flavonoids, and phenols in Spondias mombin. This study reveals the antifungal properties of the leaf extracts of Glyphaea brevis and Spondias mombin, which are commonly used in the construction of traditional yam barns in Mbaise (south-east Nigeria). The results of this study justify the exploitation of leaf extracts of Glyphaea brevis and Spondias mombin for use as an appropriate bio-alternative to existing chemical methods in the preservation of postharvest yam.

Keywords: sensitivity, fungal pathogen, dry rot, post-harvest yam, leaf extract, *Glyphaea brevis*, *Spondias mombin*.

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1. INTRODUCTION

Yams are monocotyledonous plants that belong to the genus *Dioscorea* in the family Dioscoreaceae (IITA, 2009). Yams serve as staple food for millions of inhabitants of the tropics and sub-tropics (Ezeike, 1995). The statistics for production are inconsistent; however the International Institute of Tropical Agriculture (IITA) reported that 52 million tons of yams were produced worldwide in 2007 (IITA, 2009). Ninety six percent of this came from Africa; the main producers being Nigeria with 71% of world production, Côte D'Ivoire 8.1%, Benin 4.3% and Ghana 3.5% (FAO, 2004).

According to Okigbo (2003), in Nigeria there is an estimated loss of about $0.13m per annum (which is about 10% of the total value of yam produced per annum) in storage. These losses are attributed by many researchers to rot caused by bacteria, fungi and nematodes (Amusa *et al*., 2003). The storage diseases of yam can be categorized into 3 based on the symptoms and the causal agents: dry rot, soft rot, and wet rot (Amusa and Baiyewu, 1999). Dry rot alone accounts for over 80% of postharvest yam loss in storage (Coyne *et al*., 2006). Dry rot of yam is caused by fungal pathogens, of which species of *Penicillium*, *Aspergillus*, and *Fusarium* are of high pathogenecity (Amusa and Baiyewu, 1999).

In Nigeria comprehensive treatment of post harvest yam before or during storage is not very common. This is basically due to insufficiency or the outright absence of appropriate logistics, infrastructure, funds, and skill (Amadioha and Obi, 1999). In south-east Nigeria, traditional yam barns where postharvest yams are stored and preserved till the next planting season are usually erected using live rooted growing shrubs as the main vertical framework. Yam tubers are then tied upon these shrubs using natural plant–based fibres (usually from oil palm and raffia palm leaf fronds) (Fiagan, 1995). There is a traditional belief by local farmers that yams tied to these live shrubs are much better

preserved than yams stored on the ground, improvised yam barns made of dead wood sticks, or horizontal wooden platforms like wooden planks. Different shrubs are used by different localities in the construction of traditional yam barns in south-east Nigeria. The choice of shrub varies from one locality to another, depending on the prevalent shrubs in that particular area. However two of the shrubs commonly used in the construction of traditional yam barns in Mbaise of south-east Nigeria have been chosen for this study, namely: *Glyphaea brevis* and *Spondias mombin*. The objective of this study is to test for the antifungal potencies of leaf extracts of these plants on *Aspergillus niger*, *Fusarium oxysporum*, and *Penicillium oxalicum* which have been documented by Aboagye *et al.* (2005) and other researchers to be implicated in dry rot of post-harvest yam. With the high rate of losses recorded in postharvest yam in Nigeria annually, coupled with very expensive and limited chemical treatment options, the provision of a readily available, economical, user- and eco-friendly alternative justifies the purpose of this study.

2. MATERIAL AND METHODS

Collection of samples: Dry rot infested yam tubers were collected from yam stored on dry wooden platform at Okpofe, a town in Mbaise of Imo State, Nigeria. Fresh leaves of *Glyphaea brevis* and *Spondias mombin* were collected from a traditional yam barn in the same town at about midday.

Isolation of test pathogens: Using the schemes of Okigbo and Nmeka (2005), the dry rot infested yam tubers were rinsed in sterile distilled water and then surface sterilized with 70% ethanol. The yam tubers were then cut open with a sterile knife. About 3 mm of each infected tissues was picked with sterile forceps and aseptically inoculated on sterile solid Sabouraud dextrose agar (SDA) plates. The plates were then incubated at ambient room temperature (29 – 31^{0}C) for about 5 – 7 days. The plates were examined daily for the emergence of fungal growth. The emerging colonies were isolated and sub-cultured to obtain pure cultures. The pure cultures were transferred to SDA slants and stored at 4^{o}C till required.

Identification of Test Isolates: The fungal isolates were identified using their growth (on SDA) and microscopic morphologies as described by Ogbulie *et al.* (2001) and Domsch *et al.* (1980).

Extraction of plant materials: The leaves were sun dried till constant weight and ground to powder using a mechanical grinder. For cold ethanol extraction, 50g of each powdered plant material was homogenized in 150mL of 95% ethanol and left for 48 hours, while hot ethanol extraction was carried out by homogenizing 50g of each powdered plant material in 150mL of 95% ethanol which was maintained at 60^{0}C for 1 hour in a water bath (Osadebe and Ukwue, 2004). The resulting slurries were filtered through folds of sterile cheese-cloth and the filtrates evaporated to dryness (constant weight) by forced air pressure using a rotary evaporator as described by Esimone *et al.* (1998).

Preparing plant extract diluents: Adopting the scheme of Nweze *et al.* (2004), 1000mg (1g) of each dried ethanol extract was homogenised in 1000mL of sterile distilled water to obtain a concentration of 1mg/mL stock solution. Two fold serial dilutions were used to obtain the following concentrations (diluents) in sterile distilled water: 0.5 mg/mL, 0.25mg/mL, 0.125mg/mL, and 0.0625mg/mL.

Preparation of test isolates: The test fungi were inoculated onto sterile SDA slants and incubated at ambient room temperature (29 - 31^{0}C) for 4 days in order to spur the emergence of young actively growing cultures. A portion of each fungal growth was aseptically scrapped off and placed in 10 ml sterile saline (0.9% w/v), which was shaken using a vortex mixer in order to dismember the fungal filaments (Esimone *et al.*,1998).

Determining the anti-fungal potency of plant extracts: The potencies of the plant extracts on the test fungi isolates were determined using the cup-plate agar diffusion technique. 0.2 ml of fungal suspension was aseptically introduced

into a sterile Petri-dish, and then 20 ml of cooled sterile molten SDA was added while slowly rotating to ensure uniform distribution of the microorganisms. The plates were covered, labeled, and allowed to solidify. Then 8mm diameter cups were made in the solidified agar using a sterile cork borer. 0.04mL of each serially diluted plant extracts was aseptically introduced into a separate cup, and the plates labeled according to plant extract and concentration. The dishes were then incubated at ambient room temperature (29 – 31^{0}C) for 3 – 7 days. The clear zones of growth inhibition that emerged at the end of incubation were measured using a transparent millimeter calibrated ruler. Control experiments were set up using sterile distilled water, sterile saline (0.9% w/v), and 95% ethanol.

Estimation of minimum inhibitory concentration (MIC): The MIC of the plant extracts were determined by finding the antilogarithm of the intercept on the y-axis, when the square of inhibition zone diameter was plotted against the logarithm of the concentration using Microsoft Excel 2003 software (Osadebe and Ukwue, 2004).[17]

Determination of minimum biocidal concentration: The macro broth method of Shadomy and Espineal - Ingroff (1985)[18] was used to test for minimum biocidal concentration (MBC).

Phytochemical analysis: The standard procedures described by Harborne (1973)[19], Trease and Evans (1989)[20], and Sofowora (1993)[21], were employed in carrying out Phytochemical tests.

3. RESULTS AND DISCUSSION

Fig. 1 illustrates the sensitivity of Aspergillus *niger* to *Glyphaea brevis* and *Spondias mombin* (hot and cold) ethanol extracts at different concentrations. The zones of inhibition were directly related to the concentrations evaluated. ANOVA revealed significant difference ($p<0.05$) in the sensitivity of *Aspergillus niger* to *Glyphaea brevis* and *Spondias mombin* for both cold and hot extracts.

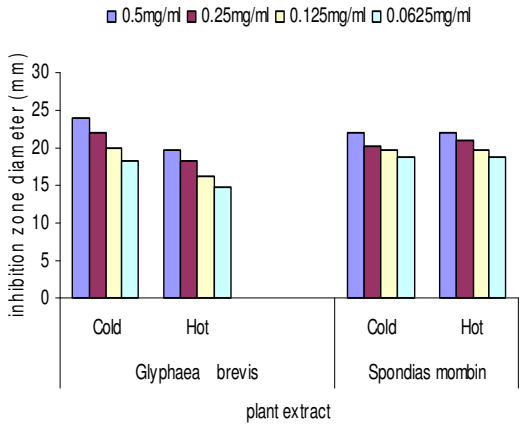

Figure 1. Relative effect of ethanol plant extracts on *Aspergillus niger*

Fig. 2 illustrates the sensitivity of Penicillium *oxalicum* to *Glyphaea brevis* and *Spondias mombin* (hot and cold) ethanol extracts at different concentrations. The zones of inhibition were directly related to the concentrations, except for the hot extract of *Spondias mombin* which showed no potency against *Penicillium oxalicum*. ANOVA revealed significant difference ($p<0.05$) in the sensitivity of *Penicillium oxalicum* to the cold extracts of *Glyphaea brevis* and *Spondias mombin*.

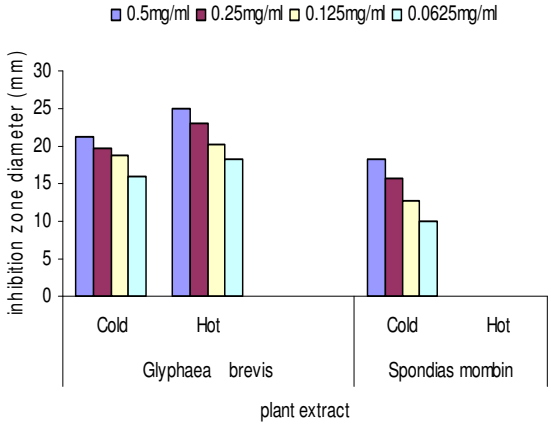

Figure 2. Relative effect of ethanol plant extracts on *Penicillium oxalicum*

Fig. 3 illustrates the sensitivity of *Fusarium oxysporum* to *Glyphaea brevis* and *Spondias*

mombin (hot and cold) ethanol extracts at different concentrations.

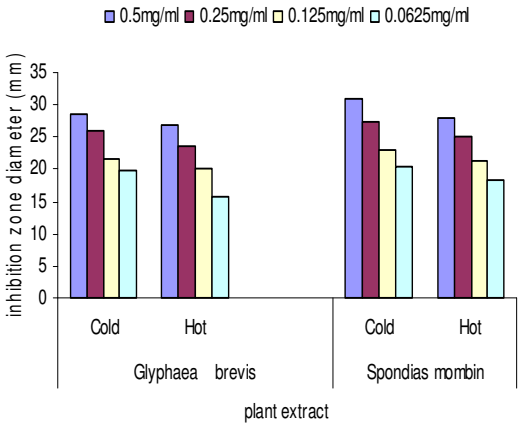

Figure 3. Relative effect of ethanol plant extracts on *Fusarium oxysporum*

The zones of inhibition were directly related to the concentrations. ANOVA revealed significant difference ($p<0.05$) in the sensitivity of *Fusarium oxysporum* to *Glyphaea brevis* and *Spondias mombin* for both cold and hot extracts.

Table 1 displays the minimum inhibitory concentrations (MIC) of each extract on a given test isolate. None of the extracts showed fungicidal effect on any of the test isolates.

Table 2 shows the different phytochemicals present in the ethanol leaf extracts of *Glyphaea brevis* and *Spondias mombin*.

The above results showed that *Glyphaea brevis* and *Spondias mombin* have antifungal potencies against *Aspergillus niger*, *Penicillium oxalicum*, *Fusarium oxysporum*. This corroborates reports by Abo and Ashidi (1998), and Dickson *et al.* (2011) that leaf extracts of *Glyphaea brevis* and *Spondias mombin* respectively displayed antimicrobial properties. Cold extracts showed on all the test isolates while the hot extracts showed potency on all but *Penicillium oxalicum.* This may be due to some of the active principles in extracts being heat labile as reported by Volk and Wheeler (1984), and Tenover (2006).

However the higher potency of hot extract of *Glyphaea mombin* on *Aspergillus niger* may on the other hand be due to a higher concentration of antifungal component(s) being extracted at higher temperature.

Computer generated ANOVA and Fisher's pair wise comparison revealed that there was a significant difference ($p<0.05$) in the potencies of *Glyphaea brevis* and *Spondias mombin* on all test isolates. ANOVA and Fisher's pair wise comparison also revealed that there were significant differences ($p<0.05$) in the effects of the different concentrations of each plant extract.

Table 1: Minimum inhibitory concentration (MIC) of ethanol extracts on test fungal isolates.

Extracts	Minimum inhibitory concentration (mg/mL)		
	Aspergillus niger	*Penicillium oxalicum*	*Fusarium oxysporum*
Glyphaea brevis (C)	2.24×10^{-3}	2.24×10^{-3}	7.08×10^{-3}
Glyphaea brevis (H)	4.0×10^{-4}	3.16×10^{-3}	1.26×10^{-3}
Spondias mombin (C)	1×10^{-4}	2.8×10^{-4}	5.01×10^{-3}
Spondias mombin (H)	1.32×10^{-4}	-	6.31×10^{-3}

(H) = Hot Ethanol Extract, (C) = Cold Ethanol Extract.

Tab 2: Phytochemical analysis of ethanol leaf extracts of *Glyphaea brevis* and *Spondias mombin*.

	Glyphaea brevis	*Spondias mombin*
Saponins	+	+
Tannins	-	+
Glycosides	+	-
Alkaloids	-	+
Flavonoids	-	+
Phenols	-	+

+ = present
- = absent

The results of the MIC tests revealed that both *Glyphaea brevis* and *Spondias mombin* were most potent on *Aspergillus niger* and least potent on *Fusarium oxysporum*. The observed antimicrobial activity of these leaf extracts may be attributed to the presence of the secondary metabolites (pyhtochemicals) as reported by Khwaja *et al*. (1996) and Ngumah (2012).

4. CONCLUSIONS

The results obtained in this work do not explicitly substantiate the folkloric belief that postharvest yams are better preserved when tied to *Glyphaea brevis* and *Spondias mombin* (when used in the construction of traditional yam barns). However, the *in vitro* experiments carried out in this study highlight the antifungal potentials of leaf extracts of *Glyphaea brevis* and *Spondias mombin,* and suggests for their exploitation in controlling post harvest rot of yam. Experiments should be carried out to see whether the antimicrobial effects of these plants are exerted on the yam tubers when tied to them. Other trials to determine *in vivo* effects of these extracts on yam tubers are recommended. The antifungal potencies of these extracts may also be tested on other pathogens implicated in postharvest yam and other crops. The specific antifungal component(s) should be isolated and determined too. This study provides data for the development of a more economical, user-, and eco-friendlier bio-alternative to existing chemical methods.

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