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ANTIFUNGAL POTENCIES OF ETHANOL LEAF EXTRACTS OF FOUR PLANTS FOUND AROUND TRADITIONAL YAM BARNS IN SOUTH EASTERN NIGERIA ON FIVE YAM PATHOGENS IMPLICATED IN POSTHARVEST YAM ROT

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ABSTRACT

The susceptibilities of five fungi implicated in postharvest rot of yams, namely, *Aspergillus niger, Penicillium oxalicum*, *Mucor circinelloides, Fusarium oxysporum,* and *Rhizopus nigricans* were evaluated using ethanol leaf extracts from four plants commonly found around traditional yam barns in South Eastern Nigeria. The plants screened were *Fagara rubescens*, *Neubouldia laevis*, *Pterocarpus soyauxii*, and *Vernonia amygdalina.* Antimicrobial susceptibility was measured as an index of turbidity of broth cultures after an incubation period of 72 hours at ambient temperature (29 – 31^{0}C). The optical densities (OD_{650}) were measured by spectrophotometry. None of the tests showed susceptibility to any of the plant extracts screened irrespective of the concentration employed. This study also revealed that although all the plants contained bioactive secondary metabolites, no antifungal potency was detected for any of the plant extracts on the test organisms.

Keywords: yam pathogens, plant extracts, yam rot, susceptibility, phytochemicals

INTRODUCTION

Yams are monocotyledonous plants belonging to the genus *Dioscorea* in the family *Dioscoreaceae*. Yams serve as a staple food for millions of inhabitants of the tropics and sub-tropics. *Dioscorea* species are important food crops in West Africa, the Caribbean, and **some** parts of Asia (including China, Japan, Malaysia and Oceania).[1]

Shrines and traditional yam barns in South Eastern Nigeria serve as home for various plants, some of which have been proven to be very valuable. Some of these plants are used for therapeutic purposes.[2] This study investigates the antifungal properties of the leaves of some of these plants found around traditional yam barns on common fungi implicated in the postharvest rot of yam. The plants screened in this study were: Fagara *rubescens* (Planch), *Neubouldia laevis* (Seem), *Pterocarpus*

soyauxii (Taub), and *Vernonia amygdalina* (Linn). These plants were chosen because they are commonly used in constructing traditional yam barns in South Eastern Nigeria, and are unofficially believed to play a role in the preservation of stored yams.

MATERIAL AND METHODS

Sample collection

Rotten yam tubes were collected from yams stored on horizontal bamboo platforms (improvised yam barns) at Okwuaba village in Okpofe, Ezinihitte Mbaise Local Government Area of Imo State (Nigeria). Fresh leaf samples were plucked at about midday at Okwuaba Okpofe. The leaves were authenticated by the Department of Forestry, Imo State Ministry of Agriculture, Owerri. The leaves were air dried at ambient room temperature (28^0C – 31^0C) until constant weight was achieved. The dried leaves were then ground to powder using a mechanical grinder.

Extraction of plant materials

70% Ethanol was used for the extraction of the plant materials. For the cold ethanol extraction, 100g of each powdered plant material was steeped in 500ml of 70% ethanol for 48 hours, while the hot ethanol extraction was carried out by seeping 100g of each powdered plant material in 500ml of 70% hot ethanol which was maintained at 60^0C for 1 hour in a water bath.[2] The slurries were filtered through folds of sterile cheese cloth. The filtrates were evaporated to dryness by forced air pressure using a rotary evaporator to a yield of about 12.5% w/w (with respect to the powdered plant material).[3]

Preparation of plant extract diluent

1000mg quantity of each ethanolic extract (hot and cold) was reconstituted with 5 ml of 10% dimethyl sulfoxide (DMSO) to obtain a concentration of 200 mg/ml.[4] A two fold serial dilution was used to obtain the following concentrations in sterile distilled water: 100 mg/ml, 50 mg/ml, 25 mg/ml, and 12.5 mg/ml. These were stored by refrigeration at 4^0C in sterile amber coloured bottles until required.

Isolation of fungal pathogens from rotten yam

The rotten yam tubers were rinsed in sterile distilled water and surface sterilized with 95% ethanol. The rotten yam tubers were then cut open with a sterile knife (flamed). About 3 pieces (3 mm diameter) of each infected tissue were picked with flamed sterilized forceps and inoculated on separate sterile solid Sabouraud's Dextrose agar (SDA) plates.[5] The plates were incubated at ambient room temperature (29 – 31^0C) for up to 5 – 7 days and examined daily for growth of moulds. The isolates were then sub-cultured to obtain pure cultures of the organisms, which were eventually transferred to SDA slants and stored at 4^0C until required.

Characterization of fungal isolates

The fungal isolates were identified using their growth (colonial) morphology on SDA and microscopic morphology. The colonial morphology on SDA was determined by observing the surface and reverse views of the fungi growing on each agar plate. The

colour, shape, elevation, and spore head colouration were noted. To view the microscopic morphology, two drops of cotton blue lactophenol were placed on a clean grease-free glass slide. Then**,** sterile (flamed) inoculating needles were used to transfer a small portion of mycelial growth to the cotton blue lactophenol. The fungal growth was then teased out in the cotton blue lactophenol and covered with a clean grease-free coverslip and then examined microscopically using x10 and x40 objectives.[6]

Preparation of inoculum

All fungal isolates were aseptically inoculated onto sterile SDA slants prepared in McCartney bottles and incubated at ambient room temperature (29 - 31^0C) for 4 days to obtain young actively growing cultures consisting of mycelia and conidia / arthrospores / blastospores. The fungal growth on each agar slant was aseptically scraped off and placed in 10 ml sterile saline (0.9% w/v) and shaken vigorously using a vortex mixer until the fungal filaments were broken into small colony forming units (cfu). Each suspension was standardized using a haemocytometer to obtain 10^4 - 10^6 cfu/ml and this was used as the inoculum.

Testing for anti-fungal potency of the plant extracts

The susceptibility of the isolated fungal pathogens to different concentrations of the plant extracts were examined using a spectrophotometer by measuring their optical density (absorbance) at 620nm.[3] 0.5 ml of the standardized fungal suspension was inoculated into a separate test tube containing 9.0 ml of sterile Sabouraud's Dextrose broth (SDB). Then**,** 0.5 ml of the reconstituted plant extract was finally inoculated into each test tube. This was carried out for the following concentrations of each plant extract: 100 mg/ml, 50 mg/ml, 25 mg/ml, and 12.5 mg/ml. The test tubes were incubated at ambient room temperature (29 – 31^0C) for 72 hours and then transferred to a spectrophotometer, where their optical densities were measured. For each set of experiments there was a control test tube containing SDB and plant extract without fungal isolate. The control was used to blank the spectrophotometer.

Phytochemical analysis

Powdered leaf plant samples were used to carry out phytochemical tests using standard procedures.[7]

RESULTS AND DISCUSSION

Table 1. Optical densities of 72 hours Sabouraud's Dextrose broth cultures of five yam (postharvest) pathogens treated with four leaf extracts**.**

Plant Extracts	Optical Density at 620nm (OD_{620}) ± SD				
	A. Niger	*P. oxalicum*	*M. circinelloides*	*F. oxysporum*	*R. nigricans*
Fagara rubescens	0.78±0.023	0.8±0.03	0.8±0.042	0.8 ±0.042	0.77±0.035
Neuboldia laevis	0.82 ±0.025	0.8±0.042	0.79±0.041	0.77±0.035	0.8±0.042
Pterocarpus soyauxii	0.77±0.031	0.78±0.046	0.8±0.042	0.82±0.012	0.8±0.042
Vernonia amygdalina	0.81±0.023	0.81±0.017	0.77±0.038	0.8±0.02	0.82±0.006
Control	0.56	0.56	0.56	0.56	0.56

Each leaf extract recorded the same optical density for all the concentrations screened per test isolate. The presence of fungal growth was confirmed using microscopy by viewing samples from the broth cultures using x10 and x40 objectives, and then plating 0.1mL on solid SDA. None of the leaf extract concentrations screened in this work showed antifungal potency on any test organism, this is at variance with the reports of researchers who demonstrated that ethanol leaf extracts of Carica papaya, Glyphaea brevis, and Spondias mombin were potent against the same test organisms used in this work (Ngumah, 2012; and Ngumah et al., 2013).[8,9]

Comparison using analysis of variance (ANOVA) showed no significant difference in the OD_{650} ($P>0.05$): within different concentrations of the same leaf extract for each test isolate, and among the different plant extracts screened on each test isolate.

Although the plant leaves screened in this work contained bioactive secondary metabolites (namely: alkaloids, tannins, and flavonoids) reported by workers to confer antimicrobial activity to plant extracts, no antifungal activity was recorded in this work by these leaf extracts.[10,11,12] This lack of antifungal activity may be attributed to: probable low concentration levels of phytochemicals,[13,14] resistance levels among strains,[15] age of leaves,[16] state of leaf at point of extraction-dry or fresh,[17,18] and method/extraction solvent**.**[19]

CONCLUSION

Although data obtained in this work revealed the presence of bioactive substances, none of the plants screened showed antifungal activity on any of the test isolates. This suggests that the mere presence of bioactive secondary metabolites does not necessarily guarantee the antimicrobial potency of plant extracts. Secondly, though there was no antimicrobial activity recorded for the test organisms, the same plant extracts may exhibit antimicrobial properties on other organisms. In addition, quantitative analysis should be done to ascertain the phytochemical levels in these extracts and compare them with levels found in other plant extracts that have been documented to exhibit antifungal activity on the same test organisms used in this work.

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