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DETERMINATION OF POTENCY OF CRUDE EXTRACTS OF *CASSIA ALATA* AND *AZADIRACHTA INDICA* AGAINST FUNGAL DETERIORATION OF SOME STORED FOOD PRODUCTS IN NIGERIA

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ABSTRACT

The paper highlights the potential of aqueous extracts of *Cassia alata* and *Azadirachta indica* as preservatives of stored foodstuffs. Different concentrations of aqueous extracts of both plants were tested in-vitro for antimicrobial activities against *Aspergillus* sp, *Mucor* sp, *Saccharomyces* sp and *Rhizopus* sp isolated from spoilt stored food commodities in Calabar, Nigeria. The minimum inhibitory concentration (MIC) study revealed, for *C alata* 62.5mg/ml against *Aspergillus* sp, Mucor spp, *Rhizopus* sp. and 31.3mg/ml against *Saccharomyces* sp. *A. indica* showed MIC of 62.5mg/ml against A*pergillus* sp, *Mucor* sp. and 31.3mg/ml against *Sacccharomyces* sp and *Rhizopus* sp. The minimum cidal concentration, on agar, of *C. alata* was 125mg/ml for all test fungi. *A. indica* showed cidal effect at 125mg/ml against *Aspergillus* sp, *Mucor* sp and 62.5mg/ml against *Saccharomyces* sp and *Rhizopus* sp. The results present the efficacy of tested plant extracts in controlling the growth of the storages fungi.

KEYWORDS

Aqueous extract, antimicrobial activity, *Cassia alata*, *Azadirachta indica*, minimum inhibitory concentration (MIC), minimum fungicidal concentration (MFC).

INTRODUCTION

The problem of the world's food supply is becoming more serious. A major contributive factor is lack of adequate food preservation and storage methods, especially in Nigeria and other developing countries (Walker, 1994). The microbial spoilage of stored food commodities depends on various factors, such as; the spoilage microorganisms, the intrinsic factors of the food, preharvest conditions, harvesting techniques and storage methods (Oke and Aro, 2001).

Fungi such as *Aspergillus niger, Botryodiplodia theobromae, Rhizopus stolonifier, Pencillum* sp and yeasts have been associated with spoilage of stored tomatoes, african breadfruit, potatoes (Nwufo *et al*, 1987)

To overcome the threat posed by spoilage fungi to the supply of food, it may be profitable that, medicinal plants with proven effectiveness against pathogenic organisms (Egwari, 1999, Hammer *et al*, 1999, Adeola *el al*, 2001, Okemo *et al* 2001) should receive consideration for their possible use as food preservatives.

Azadirachta indica (family: meliaceae) with the common name: 'neem' is known as 'Dongoyaro' in Nigeria. It is a tree planted for shade and medicinal purposes in West, East and Central Africa.

It also grows wild in India. The leaves and bark, separately or together, are used in decoction for treatment of fevers through oral administration, inhalation or hydrotherapy (Sofowora, 1982)

*Cassia alata (*family: Leguminoseae*)* A tree growing from 12 to 15m high with yellow flowers hanging in clusters. It is native to tropical regions. It can be found in all parts of Nigeria, India, southeast Asia, the west Indies and central America. The flesh of the fruit contains anthraquinonic derivatives, besides several sugars and mucilage (Pamplona-Roger, 2003). The plant has been used as a laxative in its native areas for ages.

MATERIALS AND METHODS

Plants Materials: Fresh leaves of *A. indica* and *C. alata* were collected from the botanical garden of the University of Calabar. The identification of the plant was kindly done by Dr. Deji Owolabi of the Department of Botany, University of Calabar. The leaves were washed with distilled water and dried at 60°C for 24h. in the oven after which they were ground to into a fine powder.

Extraction: Aqueous extracts of *C. citratus* and *A. indica* were prepared according to the method of Olukoya *et al* (1993). 150g of the ground leaves of each plant were soaked in 300ml distilled water in sterile beakers for four days. The extracts were clarified with sterile filter paper and filtered with Millipore filter using a vacuum pump. The extracts were concentrated in a rotary evaporator at 40°C. The concentrated extract was weighed and stored in the refrigerator at 8°C prior to use. The aqueous extract of *A. indica* yielded 24.2g and that of *C. alata* yielded 25.1g.

Preparation of Different Concentrations of Extracts.

Five grams of each aqueous extract was added to 10ml distilled water in a test tube to make 0.5g/ml (i.e. 500 mg/ml) solution. Doubling dilutions of this was done to obtain the ½(250mg/ml), ¼(125mg/ml), 1/8(62mg/ml), 1/16(31mg/ml) and 1/32(16mg/ml) dilutions.

Collection of Spoilt Food Commodities and Fungal Isolation.

Potatoes, onions, groundnuts, tomatoes and rice showing visible signs of spoilage were collected from 4 markets in the Calabar municipalities. The infected food commodities were rinsed in distilled water. The infected parts of the food commodities were sliced into separate mortars containing distilled water, which were accordingly labeled, and gently homogenized with sterile pestle. 1ml from each pestle was added to 9ml distilled water in a test tube and this was serially diluted to the 6-fold dilution. Inoculation was made into malt extract agar (MEA), impregnated with 0.5mg/ml of chloramphenicol, from the 10^{-4} and 10^{-5} dilutions(Mishra and Dubey, 1993). Two further subcultures of morphologically distinct colonies were made.

Identification of Fungal Isolates.

Macroscopic study of isolates was by visual observation of their mycelia. The needle mount technique (NMT) using lactophenol-in-cotton blue was used for the microscopic identification of the isolated fungal. Sugar fermentation test was conducted for the yeast isolate. The fungi isolated were *Aspergillus* sp, *Mucor* sp, *Saccharomyces* sp, *Rhigopus* sp.

Determination of Minimum Inhibitory concentration.

At the different concentrations of 250mg/ml, 125mg/ml, 62.5mg/ml, 31.3mg/ml and 16mg/ml of each aqueous extract, 3ml were incorporated into 9ml of potato dextrose broth in appropriately labeled test tubes. These were inoculated with each of the fungal isolates. 9 ml-potato dextrose broth in test tubes, each containing each of the fungal isolates but without plant extract served as the control. The tubes were incubated at 27°C for 48h. MICs were determined as the lowest concentration of plant extract that produced no turbidity.

Determination of Minimum Fungicidal Concentration.

A sterile swab stick was dipped into each negative tube in the MIC assays and streaked on PDA. With sterile swab sticks, broth from each control tubes in the MIC assays were streaked on PDA to serve as control. MFCs were determined as the lowest concentration of extract that produced no fungal growth on the PDA.

RESULTS AND DISCUSSION

In this study, four fungi; *Aspergillus* sp, *Mucor* sp, *Saccharomyces* sp, and *Rhizopus* sp were isolated form spoilt potatoes, groundnuts, onions, tomatoes and rice. Nwufu *et. al.* (1987) implicated *Rhizopus stolonifer* and *Aspergillus* sp in the spoilage of stored tomatoes, african breadfruit and potatoes. Oke and Aro (2001) showed that *Aspergillus* sp and *Rhizopus* sp are important rot fungi of Guava in storage.

This study shows that aqueous leaf extract of *C. alata* and *A. indica* possess antifungal activity against the test fungi.

The results in Tables 1 and 2 revealed for *C. alata, MIC* of 62.5mg/ml against *Aspergillus* sp, *Mucor* sp, *Rhizopus* sp and 31.3mg/ml against *Saccharomyces* sp and for *A. indica,* MIC of 62.5mg/ml against *Aspergillus* sp and *Mucor* sp and 31.3mg/ml against *Saccharomyces* sp and *Rhizopus* sp. Gedunin, isolated from neem has been reported to possess antifungal activity (Hoelmer *et al,* 1990). Zeringue and Bhatnagar (1994) reported the antifungal effect of *A. indica* leaf extract on growth and aflatoxin production in submerged culture of aflatoxigenic *Aspergillus parasiticus.* However previous report on the antifungal activity of *C. alata* is scanty. It was observed that higher concentrations of extracts might be required in controlling growth of *Aspergillus* sp and *Mucor* sp.

Based on the results of MFC (Table 3 and 4), it is observed that for effective control of these spoilage fungi, higher concentrations, at least 125mg/ml may be required for extract of *C. alata* and *A indica.* However, for *Saccharomyces* sp and *Rhizopus* sp, 62.5mg/ml of extracts of both plants may be adequate. This is due to difference in sensitivity of the fungi to the plant extract. The higher in-vitro activity of *A. indica* extract over *C. alata* may be attributed to differences in their bioactive components. The knowledge of these MICs and MFCs is important in deciding effective concentration of these plant extracts as preservatives of stored food commodities.

Table 1: Minimum Inhibitory concentration of Extract of *C alata against Isolated Fungi.*

Isolated fungi.	Doubling Dilutions						
	Neat	1/2 (250mg/ml)	1/4 (125mg/ml)	1/8 (62.5mg/ml)	1/16 (31.3mg/ml)	1/32 (15.6mg/ml)	Control
Aspergillus spp	-	-	-	-	+	++	+++
Mucor sp	-	-	-	-	+	++	+++
Saccharomyces sp	-	-	-	-	-	+	+++
Rhizopus sp	-	-	-	-	+	++	+++

KEY: Neat = Broth with extract without fungi.
Control = broth with Fungi and water

(-) = No growth
(+) = Little growth
++ = Average growth
+++ = Normal growth

Table 2: Minimum Inhibitory concentration of Extract of *A indica against Isolated Fungi.*

Isolated fungi	Doubling Dilutions						
	Neat	½ (250mg/ml)	¼ (125mg/ml)	1/8 (62.5mg/ml)	1/16 (31.3mg/ml)	1/32 (15.6mg/ml)	Control
Aspergillus sp	-	-	-	-	+	++	+++
Mucor sp	-	-	-	-	-	++	+++
Saccharomyces sp	-	-	-	-	-	+	+++
Rhizopus sp	-	-	-	-	-	+	+++

KEY: Neat = Broth with extract without fungi.
Control = broth with Fungi and water

(-) = No growth
(+) = Little growth
++ = Average growth
+++ = Normal growth

Table 3: Minimum Fungicidal concentration of Extract of *C alata* against Isolated Fungi.

Isolated fungi	Doubling Dilutions					
	Neat	½ (250mg/ml)	¼ (125mg/ml)	1/8 (62.5mg/ml)	1/16 (31.3mg/ml)	Control
Aspergillus sp	-	-	-	+	+	+++
Mucor sp	-	-	-	+	+	+++
Saccharomyces sp	-	-	-	+	+	+++
Rhizopus sp	-	-	-	+	+	+++

KEY: Neat = Agar medium with extract without microorganism.
Control = Agar medium with fungi and water
(-) = No growth.
(+) = Little growth.
+++ = Normal growth.

Table 4: Minimum Fungicidal concentration of Extract of *A. indica* against Isolated Fungi.

Isolated fungi	Doubling Dilutions					
	Neat	½ (250mg/m)	¼ (125mg/ml)	1/8 (62.5mg/ml)	1/16 (31.3mg/ml)	Control
Aspergillus sp	-	-	-	+	+	+++
Mucor sp	-	-	-	+	+	+++
Saccharomyces sp	-	-	-	-	+	+++
Rhizopus sp	-	-	-	-	+	+++

KEY: Neat = Agar medium with extract without microorganism.
Control = Agar medium with fungi and water.
(-) = No growth.
(+) = Little growth.
+++ = Normal growth.

The aqueous extracts of both *C. alata* and *A indica* have been shown, in this study, to be effective against spoilage fungi of stored food commodities, indicating effective diffusibility of the bio-active components in water. This study also highlights the potential of indigenous plants as veritable source of preservatives for stored food products

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