Advanced Journal of Microbiology Research Volume 2014
Available online at http://advancedresearchjournals.org/AJMR
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Full Length Research

Effect of extract of *Monodora myristica* and *Zingiber officinale* on the growth of fungi in sweet potato juice

Aderotimi Banso

Department Of Science Laboratory Technology, Federal Polytechnic, P.M.B. 55, Bida, Nigeria. E-mail: drbanrot@yahoo.co.uk.

Accepted 22 June, 2013

Aqueous extracts of *Monodora myristica* and *Zingiber officinale* were assayed for their antifungal effect on *Fusarium nivale, Rhizopus stolonifer* and *Aspergillus fumigatus* isolated from deteriorating sweet potatoes. Aqueous extracts of *M. myristica* and *Z. officinale* showed inhibitory effect against *F. nivale, R. stolonifer* and *A. fumigatus* in sweet potato juice. 3% (v/v) aqueous extract of *M. myristica* or *Z. officinale* reduced the growth of the fungi; however a combination of 2% each of both plant extracts retarded the growth better. Partial purification of the aqueous extract of *M. myristica* and *Z. officinale* showed that ethyl acetate fraction of the plant extracts exhibited the highest level of inhibition of growth of the test fungi compared with diethyl ether and n- hexane fractions. Extracts of *M. myristica* and *Z. officinale* may be important sources of preservative of root juices.

Key words: *Monodora myristica, Zingiber officinale*, antifungal, inhibitory, juice, aqueous.

INTRODUCTION

Sweet potato is one of the root crops which provide the major part of the calorie need of people in the tropics. It is a good source of Vitamin C and beta-carotene (Wolf, 1992). Sweet potato cannot be stored for very long at temperatures below 13°C. They develop chilling injury at 10°C (Ihenkoronye, 1995). Chilling leads to increase in sugar content and accelerates respiratory activity. The chilling injury can result in increased susceptibility to decay and failure to sprout. Chilling also produces such physiological effects as loss of ascorbic acid and increase in chlorogenic acid (Leistner, 1992). High level of chlorogenic acid is associated with discolouration upon exposure to air, inability to synthesize carotene and accumulation of carbon dioxide in the root during chilling.

Infection of sweet potato by fungi progresses into various stages of decay during storage. Soft rot in sweet potatoe is caused by a species of *Rhizopus* that produces soft decay which consumes the root quickly, even at 16°C (Ogundana, 1972). Surface rot and end rot are caused by species of *Funsarium* that grow slowly; it may take several weeks for the entire root to be destroyed (Wolf, 1992). Storage temperature above 16°C encourages the development of virus disease that causes the development of corky areas in susceptible varieties (Matern and Kneusel, 1988).

Loss of weight is associated with high temperature and low humidity storage, resulting in the development of pithiness in the root. At harvest, 5 - 10% of sweet potato tissue is made up of intercellular spaces. As weight losses exceed volume losses during storage, these spaces increase and eventually become visible (the root becomes pithy).

Sprouting of sweet potato occurs at temperatures above 16°C. A high relative humidity encourages growth if the temperature is high enough. Sprout growth contributes to the development of pithiness.

M. myristica seed are used as condiment in West Africa, a decoction of the seed is used to treat guinea worm infection. The seeds are used as a remedy for constipation, when mixed with palm oil. Roasted and powdered seeds of the plant are very effective in curing stomach ache. The seeds are rubbed on the forehead to cure headache (Gill, 1992).

Z. officinale (ginger) is widely associated with many traditional foods. It is used as a spice (Giese, 1994). Raw ginger is chewed to stimulate the flow of saliva and to relax congested nostrils. Ginger tea is prescribed for cough, colds and influenza (Gill, 1992). The juice of the rhizome with honey is a very efficacious remedy for cough and asthma (Okanla et al., 1990). It is recommended for ailments of digestive system, rheumatism and piles. A past of the rhizome is a local stimulant in

headache and toothache. It is also used against infective hepatitis and other forms of liver disease (Gill, 1992). The aim of this study is to investigate the effect of *M. myristicia* seeds and *Z. officinale* on the growth of three spoilage fungi: *F. nivale*, *R. stolonifer* and *A. funigatus* in potato juice.

MATERIALS AND METHODS

Plant material and microorganism

The micro-organisms used were *F. nivale, R. stolonifer* and *A. fumigatus.* The organisms were isolated from deteriorating sweet potatoes and identified by standard microbiological procedures. *M. myristicia*_and *Z. officinale* used in this study were obtained from Bida, Niger State. They were identified at Forestry Research Institute of Nigeria (FRIN), Ibadan. Fresh sweet potato roots of red skin variety were obtained from Bida, Niger State.

Preparation of extracts of *M. myristica* and *Z. officinale*

The Seeds of *M. myrsticia* and rhizome of *Z. officinale* were shade dried at ambient temperature and ground into powder. Ten grammes of ground dry seed sample of *M. myristica* and rhizome of *Z. officinale* were then soaked in 250 ml of hot (70°C) sterile water contained in two separate 500 ml capacity flasks. The flasks were plugged with cotton wool, wrapped in aluminium foil, shaken vigorously and allowed to stand in the refrigerator for 72 h. The filtrate was obtained by suction and concentrated using a water bath (BT101) at 80°C until a brown viscous residue remained (Banso and Sani, 2003)

Extraction of potato juice

Potato roots were washed and immersed in 10% hypochlorite solution for 10 min. The roots were then peeled and the juice extracted manually (Banso and Ayodele, 2005)

Determination of effect of different concentrations of plant extract on growth of fungi in potato juice

20 ml sample of the juice was introduced into 100 ml capacity flasks. Extracts of *M. myristica* and *Z officinale* were then added to give concentrations (v/v) ranging from 1 - 5%. Thereafter, they were inoculated with 1ml of aqueous suspension containing 10^{10} spores of test fungi obtained by serial dilution and incubated for 7 days at room temperature (28 ± 2°C). The developing mycelia of four replicates were subsequently recovered by filtration using pre-weighed whatman No. 1 filter paper and dried to constant weight at 70°C in a hot air oven (Banso and Ayodele, 2005). Control experiments were performed without the extracts. The weight differences were analysed by analysis of variance and Duncan Multiple Range (DMR) test.

Determination of effect of combination of plant extract on growth of fungi in potato juice

The procedure for the effect of different concentration of plant extract on the growth of fungi in potato juice was repeated using the following combination of the two plant extracts (*M. myristica* and *Z officinale*); 1:1, 1:2, 2:1, and 2:2 control flasks contain no plant extract.

Determination of antifungal effect of organic solvent soluble fractions of aqueous extract of *M. myristica and Z. officinale*

The method of Isao et al. (1992) for separation of organic compounds with a slight modification was used to determine the antifungal effect of organic solvent soluble fraction of aqueous extract of *M. myristica* and *Z. officinale*. Aqueous extract of *M. myristica* or Z. *officinale* was partitioned between water and sequentially n- hexane, diethyl ether and ethyl acetate. Each fraction was collected and allowed to evaporate to dryness using a hot plate. 20 ml of 50% concentration of the residues where inoculated with 1.0 ml aqueous suspension containing 10^6 spores of the test fungi and incubated for 7 days at room temperature (28 ± 2°C). The developing mycelia of three replicates were subsequently recovered by filtration using pre-weighed Whatman No. 1 filter paper and dried to constant weight at 70°C. Control experiments were performed without the extracts. The weight differences were analysed by analysis of variance and Duncan Multiple Range (DMR) test.

RESULTS AND DISCUSSION

The plant extracts showed inhibitory effect against *F. nivale, R. stolonifer* and *A. flavus* in sweet potato juice (Table 1) . The application of *M. myristicia* showed significant reduction of fungal biomass at 3.0% while 4.0% concentration of *Z. officinale* showed significant reduction of fungal biomass. The effect of plant extracts on micro-organisms may depend on the type as well as the medium (Obeta and Uguanyi, 1995). Spices contain phenols and essential oils, which are inhibitory to micro-organisms (Nakatani, 1994). It was reported that fat and proteins bind or solubilize phenolic compounds thereby reducing their availability for antimicrobial activity (McMance and Widdowson, 1993; McNeil and Schmidt, 1993). This may partly explain why the concentrations of the extracts used in this study were overcome by the fungi. The combination of the extracts reduced the growth of *F. nivale*, *R. stolonifer* and *A. fumigatus;* however it did not impose enough stress to stop the growth of the fungi.(Table 2)

Ethyl acetate fraction of aqueous extract of *M. myristica* and *Z. officinale* exhibited the highest level of inhibition of growth when compared with n- hexane and diethyl ether fractions (Tables 3 and 4). This may suggest the suitability of ethyl acetate for the separation of the active constituents from aqueous extracts of *M. myristica* and *Z. officinale.* The results suggest that the extracts of the plants may be important sources of preservative of root juices.

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Table 1. Inhibitory effect of extract of M. myristica and Z. officinale on the growth of fungi in sweet potato juice.

Extract (%v/v)		Biomass(mg dry weight/20 ml) ± SD		
M. myristica	*Z. officinale*	*F. nivale* n = 4	*R. stolonifer* n = 4	*A. fumigatus* n = 4
		38.5 ± 0.2	35.0 ± 0.1	40.6 ± 0.2
1.0	none	32.0 ± 0.5[a]	30.0± 0.2[a]	36.7±0.1[a]
2.0	none	26.4 ± 0.01[a]	24.5± 0.01[a]	29.5±0.1[a]
3.0 **Control**	none	20.5 ± 0.3[b]	18.6± 0.2[b]	22.6±0.2[b]
4.0	none	16.5 ± 0.5[b]	14.6± 0.1[b]	16.5±0.3[b]
5.0	none	12.5 ± 0.02[b]	10.8± 0.02[b]	13.6±0.5[b]
None	1.0	34.5 ± 0.5[a]	32.7± 0.1[a]	34.7±0.3[a]
None	2.0	29.5 ± 0.01[a]	27.5± 0.01[a]	28.0±0.2[a]
None	3.0	24.3 ± 0.1[b]	23.2± 0.03[a]	24.0±0.1[a]
None	4.0	21.5 ± 0.1[b]	18.5± 0.01[b]	19.0±0.5[b]
None	5.0	14.3 ± 0.1[b]	12.8± 0.1[b]	13.5±0.1[b]

N = Number of samples, SD = Standard deviation, control contain no *M. myristica* and no *Z. officinales,* significant level of difference from control: P > 0.05, P < 0.05

Table 2. Effect of combination of aqueous extract of M. myristica and Z. officinale on the growth of fungi in potato juice.

Extract (%v/v)		Biomass(mg dry weight/20 ml juice) ±SD		
M. myristica	*Z. officinale* n = 4	*F. nivale* N = 4	*R. stolonifer* n = 4	*A. fumigatus* n = 4
		38.5±0.2	35.0±0.1	40.6±0.2
1	1	36.5±0.2	35.0±0.1	40.6±0.1
1 **Control**	2	25.0±0.2	21.5±0.01	28.5±0.1
2	1	23.0±0.01	16.0±0.1	25.0±0.1
2	2	12.5±0.1	9.5±0.1	18.0±0.1

N = Number of samples, SD = Standard deviation, All treated cases are significantly different from control: (P < 0.05), Control contain no M. myristica and no Z. officinale

Table 3. Effect of organic solvent soluble fraction of *M. myristica* on the growth of challenge fungi in potato juice.

Test organism	Biomass(mg dry weight /20 ml juice) ± SD				
	Control	Ethyl acetate fraction n = 4	n-hexane fraction n= 4	Diethyl ether fraction n= 4	20%Dimethyl Sulphoxide n= 4
F. nivale	38.5 ± 0.02	10.5 ± 0.2	14.5 ± 0.1	12.5 ± 0.1	38.5 ± 0.1
R. stolonifer	35.0 ± 0.1	9.5 ± 0.01	13.0 ± 0.1	11.0 ± 0.01	35.0 ± 0.01
A. fumigatus	40.6 ± 0.2	13.5 ± 0.1	16.5 ± 0.1	14.5 ± 0.1	40.6 ± 0.1

N = number of samples; All treated cases are significantly different from control (P < 0.05); Control contain no *M. myristica* and no *Z. officinale*

Table 4. Effect of organic solvent soluble fractions of aqueous extract *Z. officinale* on the growth of challenge fungi in potato juice.

Test organism	Biomass(mg dry weight / 20 ml juice) ± SD				
	Control	Ethyl acetate fraction n = 4	n-Hexane fraction N = 4	Diethyl ether fraction n = 4	20% Dimethyl sulphoxide n= 4
F. nivale	38.5 ± 0.02	13.5 ± 0.2	16.5 ± 0.1	13.5 ± 0.1	38.5 ± 0.2
R. stolonifer	35.0 ± 0.1	10.5 ± 0.1	14.5 ± 0.01	12.0 ± 0.01	35.0 ± 0.1
A. fumigatus	40.6 ± 0.2	15.0 ± 0.01	18.0 ± 0.02	16.0 ± 0.1	40.6 ± 0.2

N= number of samples; All treated cases are significantly different from control (P < 0.05); Control contain no *M. myristica* and no *Z. officinale.*

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