ORIGINAL ARTICLE

Antifungal Attributes of Extracts of *Ocimum gratissimum*, *Zingiber officinale*, and *Cymbopogon citratus* on Rot Fungi of Soursop Fruit

R. N. Okigbo, R. O. Ezebo, S. C. Ugwu

Department of Botany, Faculty of Bioscience, Nnamdi Azikiwe University, Awka, Anambra, Nigeria

ABSTRACT

Purpose: The present research was conducted to determine the fungitoxic effect of ethanol and cold water extracts of Ocimum gratissimum, Zingiber officinale and Cymbopogon citratus in vitro on causative agents of soursop fruit.

Materials and Methods: The microbial pathogens obtained from the bark of soursop fruits were Rhizoctonia spp, Botryodiplodia theobromae, Corticinium salmonicolor and Penicillium spp. Ethanol and cold water extracts of the plants were prepared by adding separately 25g, 50g, 75g and 100g of the leaf powder of Ocimum gratissimum, Cymbopogon citratus and rhizome powder of Zingiber officinale into 100ml of ethanol and cold water respectively.

Results: The percentage occurrence of Rhizoctonia spp was the highest (50%) while that of Penicillium spp was the lowest (10%). The pathogenicity test of fungal isolates on healthy soursop fruits revealed that Rhizoctonia spp showed highest infection diameter of 35.0 + 6.25mm followed by Botryodiplodia theobromae with infection diameter of 30.0 + 5.33mm while Penicillium spp gave the least infection diameter of 20.0 + 4.52mm.

Discussion: The plant extracts of Ocimum gratissimum, Zingiber officinale and Cymbopogon citratus in both aqueous and ethanol exhibited inhibitory activity against the isolated fungi. The activity of the plant extract in ethanol solvent increased with concentrations of the extract but varied in aqueous solvent. The highest activity of lemongrass was observed in aqueous solvent while that of scent leaf was observed in ethanol solvent. The susceptibility of fungal isolates was found to vary significantly with type of extract and with solvent of extraction.

Conclusion: This study indicated that Ocimum gratissimum, Zingiber officinale and Cymbopogon citratus were able to suppress rot-causing fungi of soursop fruits.

Key words: Annona muricata, fruit, antifungal, attributes, Cymbopogon citratus, extract, Ocimum gratissimum, Zingiber officinale

INTRODUCTION

Soursop (*Annona muricata* L.) belongs to the family Annonaceae and indigenous to tropical North and South America.[1] In Nigeria, the plant is restricted to the rainforest zones of Nigeria and cultivated mainly in home gardens in Abia, Imo, Enugu, Rivers, Ebonyi, and Anambra and Delta States. Soursop tree is a small straggly fruit tree growing up to 8 m high.[2] The flesh is pulpy white, stringy and sour containing shiny black seeds.[3]

Besides serving as a source of food, soursop has many therapeutic properties; the juice is diuretic while the other parts have antibacterial, anticancerous, astringent, sedative,

Address for correspondence:
R. O. Ezebo, Department of Botany, Faculty of Bioscience, Nnamdi Azikiwe University, P.M.B. 5025, Awka, Anambra, Nigeria. E-mail: esau_056@yahoo.com

and other properties.[4] The leaves had been traditionally used to treat headaches, hypertension, cough, asthma and used as antispasmodic, sedative, and nervine for heart condition.[5,6]

Meanwhile, there is evidence from earlier works that several plant species possess antifungal and antibacterial properties.[7-12] The extracts of *Ocimum gratissimum* has been reported to have antimicrobial properties.[13,14] Similar studies by[15] also reported that an extract of *O. gratissimum* significantly reduced radial (mycelia) growth of *Pythium aphanidermatum.* Lemongrass (*Cymbopogon citratus* L.) oil has also been reported to show antifungal activities against several plant pathogens while *Zingiber officinale* was reported to significantly control fungal rot pathogens of yam[16] and also in the control of microorganisms causing deterioration of yam chips over *Vernonia amygdalina in vitro*.[17] Moreso, application of extracts of *Z. officinale* have been reported to inhibit the mycelial growth and sporulation of *Colletotrichum capcici* (*in vitro*) the causal organism of brown blotch disease of cowpea.[18]

This study investigated the antifungal properties of leaf extracts of *O. gratissimum*, *Z. officinale,* and *C. citratus* against some spoilage fungi responsible for the deterioration of soursop fruit.

MATERIALS AND METHODS

Location

This study took place at the Plant Pathology Laboratory of National Root Crops Research Institute, Umudike, Abia State. Umudike is located on Latitude 5° 29' N and Longitude 7° 33' E in the rainforest zone of Nigeria.

Collection of plant samples

Soursops (*A. muricata*) with symptoms of post-harvest rot were bought from the markets in Umudike, Abia State in Nigeria. Fresh, healthy soursops were collected from the same market. The leaves of *O. gratissimum* and *C. citratus* were collected from farmland in Umudike, Abia State while fresh rhizomes of *Z. officinale* were purchased from Central Market Umuahia, Abia State. The botanical identities of the plants were authenticated in the Herbarium, Department of Botany, University of Agriculture, Umudike, Abia State.

Preparation of culture media

The culture media that were used for fungal growth and maintenance is potato dextrose agar (PDA). The PDA was prepared according to manufacturer's recommendation by dissolving 39 g of PDA powder in 1 L of distilled water in a 1000 ml round bottom flask. It was swirled and boiled to melt in a heater. It was sterilized with an autoclave at a temperature of 121°C for 15 min. The medium was allowed to cool to 47°C after which was poured into sterile plates (Petri dishes) and allowed to solidify.

Isolation of fungi from rotten soursops

The Petri dishes were inoculated with soursop samples by cutting sections of approximately 4 mm pieces from the tissue at the junction between a healthy and infected portion of soursops with the surface sterilized blade. They were surface sterilized (to remove surface contaminants) in 70% ethanol and then rinsed twice (1 min each) in sterile distilled water. The cut pieces of soursops were placed on sterile paper towels in a Laminar Airflow Hood Chamber for 10 min to dry and then placed onto PDA. The plates were incubated at 25°C for 4 days and then examined daily for the development of fungi growth. All plates were replicated.

Sub culturing/purification and identification of test fungi pathogens

When growth has established, subcultures were prepared using inoculate from different organisms in the mixed cultures to obtain a pure culture. This was done by transferring hyphal tips from the colony edge of the mixed cultures to fresh plates of PDA using flamed sterilized blades. After sub-culturing, the plates were incubated at 25°C until pure cultures were obtained. The Petri dishes of pure cultures of the test fungi were then sealed with paraffin to prevent contamination. The resulting pure cultures were used for characterization, and subsequent identification of the fungi isolates with the aid of a compound microscope and identification guides.[19,20]

Pathogenicity test

Fresh, healthy soursops were washed with sterile distilled water twice. Thereafter, the fruits were sterilized with 70% ethanol and washed with sterile water. The washed fruits were placed on sterile paper towels and allowed to dry for 12 min in a laminar airflow hood. A sterile 5mm cork borer was used to bore holes in the healthy soursops. The parts of the soursops which were bored out at each point were kept in sterile dishes. An agar block measuring 4mm by 4mm from growing cultures of each test isolates (pure cultures) was inoculated into the hole made with the aid of another cork borer (4 mm diameter). After inoculation, the parts of the fruit bored out were carefully replaced and sealed with sterile blue seal Vaseline to prevent contamination and labeled accordingly. A control experiment which had no isolate was set up (inoculated with agar plug alone). After inoculating the entire test isolates into their respective healthy soursops, all the soursops were incubated in a moisture chamber at 25°C for 5 days. The fruits were examined daily for evidence of rot such as softening, discoloration n, and offensive odor. At the end of the 5 days incubation period, the fruits were cut open to expose inner regions of the fruits which were examined for rot. The length and girth of the rotted area and those of the entire fruits were measured with a transparent ruler and recorded.

Preparation of plant extracts

The fresh leaves of *O. gratissimum*, *C. citratus,* and rhizome

of *Z. officinale* and were thoroughly washed with tap water and then with sterile distilled water and were sundried for 5 days for milling. The dried samples were separately ground in a Laboratory Mill (Thomas Wiley model ED-5 made in the USA) after which the ground samples were sieved to obtain powdered processed sample used for extraction. Using cold solvent extraction method,[21-23] 25 g, 50 g, 75 g, and 100 g portions of each processed samples were mixed with 100 ml of each solvent (aqueous and ethanol) separately in a bottle to produce 25%, 50%, 75%, and 100% extract concentrations, respectively. The extracts were sieved through with four layers of sterile cheesecloth and stored in sterile conical flasks which were later used for mycelia growth inhibition.

Effect of plant extracts on fungal growth

Effect of plant extract on mycelia growth with test fungi was studied using food poisoning techniques.[24] 1 ml of each plant extract concentrations (25%, 50%, 75%, and 100%) was dispensed per Petri dish, and 9 ml of the media (molten PDA) was added to each of the Petri dish containing extract and carefully spread evenly over the plate. These were used for inhibition of mycelial growth. The plates were gently rotated to ensure even dispersion of the extracts. The agar extract mixture was allowed to solidify and then inoculated at the center with a 4mm diameter mycelia disc obtained from the colony edge of 7-day-old pure cultures of test fungi. Each treatment was duplicated. The control set up consists of blank agar plate (no extract) inoculated with the test fungi as described above. All the plates were incubated at 27 ± 2°C for 5 days and examined daily for growth and presence of inhibition. Colony diameter was taken as the mean growth along two directions on two pre-drawn perpendicular lines on the reverse side of the plates. The effectiveness of the extract was recorded in terms of percentage inhibition, which was calculated according to the method described by.[25]

$$\text{Percentage inhibition} = \frac{R1\text{-}R2}{R1} \times 100$$

Where: R1: Radial distance of pathogen in control plates and R2: Radial distance of pathogen in extract incorporated agar plates.

The percentage of inhibition was determined as a guide for selecting the minimum inhibitory concentration that will be effective in controlling rot-causing fungi. Extracts were rated for their inhibitory effects using the scale described by Sangoyoni;[24] 0% inhibition: Not effective, >0–20%: Slightly effective, >20–50%: Moderately effective, >50–<100%: Effective, and 100% inhibition: Highly effective.

Statistical analysis

Data were analyzed using analysis of variance through Statistical Analysis System of Version 9.1 and means of treatment were compared using Duncan multiple range test at $P < 0.05$.

RESULTS

Percentage occurrence of fungal isolates associated with rot of soursop fruits

Fungal isolates associated with deterioration of *A. muricata* (soursop) fruits were *Rhizoctonia* spp., *B. theobromae*, *Corticium salmonicolor,* and *Penicillium* spp. The results depicted that *Rhizoctonia spp.* was the most prevalent (50%), while the least prevalent was *Penicillium* spp. (10%) [Table 1].

Pathogenicity test

The pathogenicity test of fungal isolates on healthy *A. muricata* (soursop) fruit revealed that *Rhizoctonia spp.* was the most virulent with infection diameter of 35.0 ± 6.25 mm followed by *B. theobromae* with infection diameter of 30.0 ± 5.33 mm while *Penicillium spp.* gave the least infection diameter of 20.0 ± 4.52 mm [Table 2].

Comparison of the antifungal activity of plant extracts

At 25% concentration, the results revealed that scent leaf had the highest antifungal activity than other plant extracts with percentage inhibition of 77.50 ± 0.057% for *Rhizoctonia spp.,* 87.50 ± 0.141% for *B. theobromae,* 80.00 ± 0.170% for *Penicillium* spp., and 75.00 ± 0.014% for *C. salmonicolor* while lemongrass showed lowest antifungal activity against the fungal isolates with percentage inhibition of 12.50 ± 0.042% for *Rhizoctonia* spp., 2.00 ± 0.000% for *B. theobromae,* 16.67 ± 0.000% for *Penicillium spp.,* and 2.00 ± 0.000% for *C. salmonicolor*. The inhibition of all the isolates showed a significant difference at $P < 0.05$ [Table 3].

At 50% concentration, the results revealed that scent leaf showed highest antifungal activity than other plant extracts with

Table 1: Percentage frequency of fungi isolated from soursop rot

	Species of fungi	Frequency (%)
1	*Botryodiplodia theobromae*	30
2	*Corticium salmonicolor*	25
3	*Penicillium* spp.	10
4	*Rhizoctonia* spp.	50

Table 2: Pathogenicity test of fungal isolates on soursop rot

	Species of fungi	Infection diameter (mm)
1	*Botryodiplodia theobromae*	30.0±5.33
2	*Corticium salmonicolor*	25.0±5.21
3	*Penicillium* spp.	20.0±4.52
4	*Rhizoctonia* spp.	35.0±6.25

complete inhibition of *Rhizoctonia* spp., *Penicillium* spp., and *C. salmonicolor* and a percentage inhibition of 95.00 ± 0.000% for *B. theobromae* while lemongrass showed lowest antifungal activity against the fungal isolates with percentage inhibition of 17.50 ± 0.014% for *Rhizoctonia* spp., 5.00 ± 0.283% for *B. theobromae,* 5.00 ± 0.283% for *Penicillium* spp., and 2.00 ± 0.000% for *C. salmonicolor*. The inhibition of all the isolates showed a significant difference at $P < 0.05$ [Table 4].

At 75% concentration, the results revealed that scent leaf showed highest antifungal activity than other plant extracts with complete inhibition of all fungal isolates. Lemongrass showed lowest antifungal activity against *Rhizoctonia* spp. (22.50 ± 0.014), *Penicillium* spp. (58.33 ± 0.042), and *C. salmonicolor* (2.00 ± 0.000). The inhibition of all the isolates showed a significant difference at $P < 0.05$ [Table 5].

At 100% concentration, the results revealed that scent leaf showed highest antifungal activity than other plant extracts with complete inhibition of all fungal isolates. Lemongrass showed lowest antifungal activity against *B. theobromae* (12.50 ± 0.042), *Penicillium* spp. (75.00 ± 0.198), and *C. salmonicolor* (20.00 ± 0.057). The inhibition of all the isolates showed a significant difference at $P < 0.05$ [Table 6].

At 25% concentration, the results revealed that lemongrass showed highest antifungal activity than other extracts against *Rhizoctonia spp.* (5.00 ± 0.467), *Penicillium* spp. (66.67 ± 0.071), and *C. salmonicolor* (73.33 ± 0.014). Scent leaf showed lowest antifungal activity against *Penicillium* spp. (25.00 ± 0.467) and *C. salmonicolor* (2.00 ± 0.000) while ginger extract showed the highest antifungal activity against *B. theobromae* (2.50 ± 0.014). The inhibition of all the isolates showed a significant difference at $P < 0.05$ [Table 7].

Results of the antifungal activities at 50% concentration revealed that lemongrass showed the highest antifungal activity than other extracts against *Rhizoctonia* spp. (62.50 ± 0.283), *B. theobromae* (70.00 ± 0.141), *Penicillium* spp. (66.67 ± 0.028), and *C. salmonicolor* (80.00 ± 0.028). Scent

Table 3: Antifungal activity of ethanol extracts of *Ocimum gratissimum, Zingiber officinale*, and *Cymbopogon citratus* against fungi isolates at 25% concentrations

Plant extract	Fungal isolates			
	***Rhizoctonia* spp.**	***B. theobromae***	***Penicillium* spp.**	***C. salmonicolor***
Ginger	25.00±0.707[b]	2.50±0.028[b]	50.00±0.311[b]	16.67±0.000[b]
Scent leaf	77.50±0.057[a]	87.50±0.141[a]	80.00±0.170[a]	75.00±0.014[a]
Lemongrass	12.50±0.042[c]	2.00±0.000[c]	16.67±0.000[c]	2.00±0.000[c]

Values in same column with same superscript are not significantly different ($P<0.05$) DMRT. *B. theobromae*: *Botryodiplodia theobromae*, *C. salmonicolor: Corticium salmonicolor*

Table 4: Antifungal activity of ethanol extracts of *Ocimum gratissimum, Zingiber officinale*, and *Cymbopogon* citratus against fungi isolates at 50% concentrations

Plant extract	Fungal isolates			
	***Rhizoctonia* spp.**	***B. theobromae***	***Penicillium* spp.**	***C. salmonicolor***
Ginger	25.00±0.141[a]	5.00±0.424[b]	75.00±1.697[a]	16.67±0.014[a]
Scent leaf	-	95.00±0.000[a]	-	-
Lemongrass	17.50±0.014[b]	5.00±0.283[b]	5.00±0.283[b]	2.00±0.000[b]

Values in the same column with the same superscript are not significantly different ($P<0.05$) DMRT. *B. theobromae: Botryodiplodia theobromae, C. salmonicolor: Corticium salmonicolor*, DMRT: Duncan's multiple range test

Table 5: Antifungal activity of ethanol extracts of *Ocimum gratissimum, Zingiber officinale,* and *Cymbopogon citratus* against fungi isolates at 75% concentrations

Plant extract	Fungal isolates			
	***Rhizoctonia* spp.**	***B. theobromae***	***Penicillium* spp.**	***C.salmonicolor***
Ginger	25.00±0.424[a]	2.50±0.028[b]	75.00±0.820[a]	25.15±0.537[a]
Scent leaf	-	-	-	-
Lemongrass	22.50±0.014[b]	7.50±0.000[a]	58.33±0.042[b]	2.00±0.000[b]

Values in the same column with the same superscript are not significantly different ($P<0.05$) DMRT. *B. theobromae: Botryodiplodia theobromae, C. salmonicolor: Corticium salmonicolor*, DMRT: Duncan's multiple range test

leaf showed lowest antifungal activity against *B. theobromae* (2.00 ± 0.000) and *C. salmonicolor* (25.00 ± 0.170) while ginger extract showed lowest antifungal activity against *Penicillium* spp. (45.00 ± 0.042). The inhibition of all the isolates showed a significant difference at $P < 0.05$ [Table 8].

At 75% concentration, it was revealed that ginger extract showed the highest antifungal activity than other extracts against *Rhizoctonia* spp. and *B. theobromae* while lemongrass showed highest antifungal activity against *Penicillium* spp. (80.00 ± 0.141) and *C. salmonicolor* (93.33 ± 0.467). Scent leaf showed lowest antifungal activity against *Rhizoctonia* spp. (2.00 ± 0.000), *B. theobromae* (2.00 ± 0.001), and *C. salmonicolor* (60.00 ± 0.028). The inhibition of all the isolates showed a significant difference at $P < 0.05$ [Table 9].

At 100% concentration, it was revealed that ginger extract showed highest antifungal activity than other extracts against *Rhizoctonia* spp. and *B. theobromae* while lemongrass showed highest antifungal activity against *Penicillium* spp. (86.67 ± 0.014). Scent leaf showed lowest antifungal activity against *Rhizoctonia* spp. (2.00 ± 0.000), *B. theobromae* (2.00 ± 0.001), and *C. salmonicolor* (80.00 ± 0.000). The inhibition of all the isolates showed a significant difference at $P < 0.05$ [Table 10].

DISCUSSION

In general, *Annona* fruits are susceptible to pathogenic attack due to their low pH, high moisture content, and nutrient composition. These make them rot and unfit for consumption due to the production of mycotoxins.[26] This study showed that the fungal isolates associated with *A. muricata* (soursop) fruit rot include *Rhizoctonia* spp., *B. theobromae*, *C. almonicolor,* and *Penicillium* spp. The percentage occurrence of *Rhizoctonia* spp. was the highest (50%) while that of *Penicillium* spp. was the least (10%). *Rhizoctonia* spp. and *B. theobromae* have been reported to be one of the most important fruit rot pathogens in Nigeria.[27]

Table 6: Antifungal activity of ethanol extracts of *Ocimum gratissimum, Zingiber officinale*, and *Cymbopogon citratus* against fungi isolates at 100% concentrations

Plant extract	fungal isolates			
	***Rhizoctonia* spp.**	***B. theobromae***	***Penicillium* spp.**	***C. salmonicolor***
Ginger	12.50±0.156^{b}	24.50±0.410^{a}	80.00±0.028^{a}	66.67±0.071^{a}
Scent leaf	-	-	-	-
Lemongrass	25.00±0.170^{a}	12.50±0.042^{b}	75.00±0.198^{b}	20.00±0.057^{b}

Values in the same column with the same superscript are not significantly different (P<0.05) DMRT. *B. theobromae: Botryodiplodia theobromae, C. salmonicolor: Corticium salmonicolor*, DMRT: Duncan's multiple range test

Table 7: Antifungal activity of aqueous extracts of *Ocimum gratissimum, Zingiber officinale*, and *Cymbopogon citratus* against fungi isolates at 25% concentrations

Plant extract	Fungal isolates			
	***Rhizoctonia* spp.**	***B. theobromae***	***Penicillium* spp.**	***C. salmonicolor***
Ginger	2.00±0.000^{b}	2.50±0.014^{a}	45.00±0.778^{b}	46.67±0.014^{b}
scent leaf	2.00±0.000^{b}	2.00±0.000^{b}	25.00±0.467^{c}	2.00±0.000^{c}
Lemongrass	5.00±0.467^{a}	2.00±0.000^{b}	66.67±0.071^{a}	73.33±0.014^{a}

Values in the same column with the same superscript are not significantly different (P<0.05) DMRT. *B. theobromae: Botryodiplodia theobromae, C. salmonicolor*. *Corticium salmonicolor*, DMRT: Duncan's multiple range test

Table 8: Antifungal activity of aqueous extracts of *Ocimum gratissimum*, Zingiber *officinale, and Cymbopogon citratus* against fungi isolates at 50% concentrations.

Plant extract	Fungal isolates			
	***Rhizoctonia* spp.**	***B. theobromae***	***Penicillium* spp.**	***C. salmonicolor***
Ginger	2.00±0.000^{b}	5.00±0.467^{b}	45.00±0.042^{c}	46.67±0.000^{b}
Scent Leaf	2.00±0.000^{b}	2.00±0.000^{c}	50.00±1.612^{b}	25.00±0.170^{c}
Lemongrass	62.50±0.283^{a}	70.00±0.141^{a}	66.67±0.028^{a}	80.00±0.028^{a}

Values in the same column with the same superscript are not significantly different (P<0.05) DMRT. *B. theobromae: Botryodiplodia theobromae, C. salmonicolor: Corticium* salmonicolor, DMRT: Duncan's multiple range test

Table 9: Antifungal activity of aqueous extracts of *Ocimum gratissimum, Zingiber officinale, and Cymbopogon citratus* against fungi isolates at 75% concentrations.

Plant extract	Fungal isolates			
	Rhizoctonia spp.	B. theobromae	Penicillium spp.	C. salmonicolor
Ginger	-	-	60.00±0.042[c]	86.67±0.042[b]
scent leaf	2.00±0.000[b]	2.00±0.001[b]	65.00±0.156[b]	60.00±0.028[c]
Lemongrass	75.00±1.697[a]	80.00±0.028[a]	80.00±0.141[a]	93.33±0.467[a]

Values in the same column with the same superscript are not significantly different (P<0.05) DMRT. *B. theobromae: Botryodiplodia theobromae, C. salmonicolor: Corticium salmonicolor*, DMRT: Duncan's multiple range test

Table 10: Antifungal activity of aqueous extracts of *Ocimum gratissimum, Zingiber officinale, and Cymbopogon citratus* against fungi isolates at 100% concentrations

Plant extract	Fungal isolates			
	Rhizoctonia spp.	*B. theobromae*	*Penicillium* spp.	*C. salmonicolor*
Ginger	-	-	75.00±0.000[c]	-
Scent leaf	2.00±0.000[b]	2.00±0.000[b]	80.00±0.141[b]	80.00±0.000
Lemongrass	75.00±0.820[a]	87.50±0.000[a]	86.67±0.014[a]	-

Values in the same column with the same superscript are not significantly different (P<0.05) DMRT. *B. theobromae: Botryodiplodia theobromae, C. salmonicolor: Corticinium salmonicolor*, DMRT: Duncan's multiple range test

The pathogenicity test of fungal isolates on healthy *A. muricata* (soursop) fruit revealed that *Rhizoctonia* spp. reported highest infection diameter of 35.0 ± 6.25 mm followed by *B. theobromae* with infection diameter of 30.0 ± 5.33 mm while *Penicillium* spp. gave the least infection diameter (20.0 ± 4.52 mm). *B. theobromae* has also been associated with the pre-harvest deterioration of soursop.[28]

The plant extracts of ginger, scent leaf, and lemongrass in both aqueous and ethanol solvents were shown to exhibit inhibitory activity against the isolated fungi. The results of the present study support the folkloric usage of the plants extract and show that some of the plant extracts possess compounds with antimicrobial properties and that can be used as a botanical sin the control of rot in soursop fruit. The antifungal of leaf extracts *O. gratissimum* on spore germination and mycelia reduction of the most commonly occurring fungal pathogen causing soft rot of yam tuber has been demonstrated by Okigbo and Ogbonnaya.[27] The use of lemongrass for controlling postharvest disease of several fruits has also been reported.[29]

The aqueous extract of lemongrass and ethanol extract of scent leaf showed the highest inhibitory activity against the fungal isolates when compared to other extracts. Moreover, the antifungal activity of the plant extract was found to differ significantly with solvent. In ethanol solvent, scent leaf extract showed the highest inhibitory activity against the fungal isolates for most observation while lemongrass showed the lowest activity. In aqueous solvent, the reverse was the case as lemongrass extract showed the highest inhibitory activity against the fungal isolates for most observation while scent leaf extract showed the lowest activity. The difference between the activities of plant extract has been explained by the quantity of the active compounds.[30] Furthermore Siripongvutikorn *et al*.,[31] have explained that one important factor of solvent effectiveness is the active components of the plants and that if most of the compounds contained in the plants are miscible in water, then an antimicrobial activity can be observed.

The result also showed that the inhibitory activity of the plant extracts was significantly affected by concentrations of the extract. In ethanol extracts, antifungal activity increased with increasing concentration of the extract while those of aqueous extracts varied. For instance, in scent leaf aqueous extract, the inhibitory activity against *Penicillium* spp. and *C. salmonicolor* increased with the concentration of the extract while *B. theobromae* and *Rhizoctonia spp.* remained insensitive to increasing concentrations of the extract. Similarly, an aqueous extract of lemongrass, the highest inhibitory activity occurred at 75% concentration instead of 100%. Although[32] explain that the activity of plant extracts increases with concentration of extract due to a higher quantity of active ingredients,[33] studies have shown that in water, concentration may have variable effect. According to Afolayan and Aliero[33] this is because water is considered to have large dipole molecules and a high dielectric constant, making it very polar and only miscible in itself. This is based on the compounds' miscibility in water, some extract will have antimicrobial action and some will not, even with higher concentrations.

CONCLUSION AND RECOMMENDATIONS

Results of this investigation have identified *Rhizoctonia spp.*, *B. theobromae*, *C. salmonicolor,* and *Penicillium spp.* as fungi associated with rot of soursop fruit. *B. theobromae* was found to be most virulent while *Penicillium spp.* was found to be less virulent. The result of this work showed that plant extract of *O. gratissimum, Z. officinale,* and *C. citratus* leaf was able to inhibit the mycelial growth of isolated fungi. Ethanol extract of *O. gratissimum* and aqueous extract of *C. citratus* demonstrated greater potential for the control fruit rot in soursop and therefore is recommended for the control of rot in soursop fruit.

REFERENCES

1. Samson JA. Tropical Fruits. London: Longman; 1980. p. 196-204.
2. Dupriez V, De Leener P. African Gardens and Orchards: Growing Vegetables and Fruits. London and Basingstoke: Macmillan Publishers Ltd.; 1989. p. 286-7.
3. Akubor PI, Egbekun MK. Chemical composition, physical and sensory characteristics of echekala (*Spondias mombin*) fruit juice. Niger J Nutr Sci 2007;28:1-8.
4. Asprey GF, Thornton P. Medicinal plants of Jamaica. West Indian Med J 1995;4:69-92.
5. Taylor L. Technical Data Report for Graviola, *Annona muricata*. Vol. 10. Austin: Sage Press; 2002. p. 1-6.
6. Lans CA. Ethnomedicines used in Trinidad and Tobago for urinary problems and diabetes mellitus. J Ethnobiol Ethnomed 2006;2:45-55.
7. Ali-Shtayeh MS, Ghdeib SI. Antifungal activity of plant extracts against dermatophytes. Mycoses 1999;42:665-72.
8. Ficker CE, Arnason JT, Vindas PS, Alvarez LP, Akpagana K, Gbéassor M, *et al.* Inhibition of human pathogenic fungi by ethnobotanically selected plant extracts. Mycoses 2003;46:29-37.
9. Erdogrul OT. Antibacterial activities of some plant extracts used in folk medicine. Pharm Biol 2002;40:269-73.
10. Maji MD, Chattopadhyay S, Kumar P, Saratchandra B. *In vitro* screening of some plant extracts against fungal pathogens of mulberry (*Morus* spp.). Arch Phytopathol Plant Prot 2005;38:157-64.
11. Kowalski R, Kedzia B. Antibacterial activity of *Silphium perfoliatum* extracts. Pharm Biol 2007;45:494-500.
12. Sardari S, Amin G, Micetich RG, Daneshtalab M. Phytopharmaceuticals Part 1. antifungal activity of selected Iranian and Canadian plants. Pharm Biol 1998;36:180-8.
13. Ejechi BO, Akpomedaye DE. Wood Protection Potentials of *Acalypha hispida* (*Euphorbiaceac)* Leaf Phenolic Extract against Degradation by Two Basidiomycetes. Book of Abstract, 27th Annual Conference of Nigerian Society for Plant Protection at Nigerian Institute for oil palm Research: Benin City; 1999. p. 35.
14. Owolade BF, Osinkanlu YO. Evaluation of some of plant extracts for the control of brown blotch disease of cowpea in South West Nigeria. J Sustain Agric Environ 1999;1:198-202.
15. Oluma HO, Garba IU. Screening of *Eucalyptus globules* and *Ocimum gratissimum* against *Pythium aphanidermatum*. Niger J Plant Prot 2004;21:109-14.
16. Okigbo RN, Nmeka IA. Control of yam tuber rot with leaf extracts of *Xylopia aethiopica* and *Zingiber officinale*. Afr J Biotechnol 2005;4:804-7.
17. Okigbo RN, Anukwuorji CA, Eguae CT. Control of microorganism causing the deterioration of yam with *Vernonia amygdalina* (L) and *Zingiber officinale.* Niger J Mycol 2012;4:45-56.
18. Ajayi AM, Olufolaji DB. The biofungal attributes of some plant extracts on *Colletotrichum capsicum,* the fungal pathogen of brown blotch disease of cow pea. Niger J Mycol 2008;1:59-65.
19. Sulton BC. The Coelomycetes fungi Imperfecti with Pycinidia acervuli and stromata. Kew, Surrey, England: Common Wealth Mycological Institute; 1980. p. 696.
20. Barnett HL, Hunter BB. Illustrated General of Imperfect Fungi. 4th ed. Minneapolis: Burgress Publishing Company; 1987. p. 218.
21. Harbone JB. Phytochemical Methods. London: Chapman and Hall, Ltd.; 1973. p. 49-188.
22. Junaid SA, Olabode AO, Owuliri FC, Orkworlu AE, Agina SE. The anti-microbial properties of *Ocimum gratissimum* extract on some selected bacterial gastrointestinal isolates. Afr J Biotechnol 2006;5:2315-21.
23. Doughari JH, Elmahmood AM, Manzara S. Studies on the antibacterial activity of root extracts of *Carica papaya* L. A J Microbiol Res 2007;1:37-41.
24. Sangoyoni TE. Post- Harvest Fungal Deterioration of Yam (Dioscorea rotundata Poir) and its Control. Thesis. Nigeria: International Institute of Tropical Agriculture. Ibadan; 2004. p. 179.
25. Whips JM. Effects of media on growth interactions between a range of soil-borne grass pathogens and antagonistic fungi. N Pathol 1987;107:127-42.
26. Stinson EE, Osman SF, Heisler EG, Siciliano J, Bills DD. Mycotoxin production in whole tomatoes, apples, oranges and lemons. J Agric Food Chem 1981;29:790-2.
27. Okigbo RN, Ogbonnaya UO. Antifungal effects of two tropical plant leaf extracts (*Ocimum gratissimum* and *Aframomum melegueta*) on postharvest yam (*Dioscorea* spp) rot. Afr J Biotechnol 2006;5:727-31.
28. Morton J. Fruits of Warm Climates. 2nd ed. Winterville, NC: University of Florida; 2000. p. 401.
29. Williams LR, Stockley JK, Yan W, Home VN. Essential oils with high antimicrobial activity for therapeutic use. Int J Aromather 1998;8:30-40.
30. Al-Zubaydi SR, Al-Hmdany MA, Raesan SJ. Antibacterial effect of some medicinal plant extracts against some pathogenic bacteria strains. J Duhok Univ 2009;12:244-9.
31. Siripongvutikorn S, Thummaratwasik P, Huang Y. Antimicrobial and antioxidant effects of Thai seasoning, Tom-Yum. J Food Sci Technol 2005;38:347-52.
32. Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oil – A review. Food Chem Toxicol 2008;46:446-75.
33. Afolayan AJ, Aliero AA. Antimicrobial activity of *Solanum tomentosum.* Afr J Biotechnol 2006;5:369-72.

How to cite this article: Okigbo RN, Ezebo RO, Ugwu SC. Antifungal Attributes of Extracts of *Ocimum gratissimum*, *Zingiber officinale,* and *Cymbopogon citratus* on Rot Fungi of Soursop Fruit. Clin J Nutr Diet 2018;1(1):1-7.