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Antimicrobial Properties and Phytochemical Analysis of Methanolic Extracts of Aframomum Melegueta and Zingiber Officinale on Fungal Diseases of Tomato Fruit.

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Abstract
The antimicrobial properties of methanolic extracts of Aframomum melegueta seeds and rhizomes of Zingiber officinale were investigated on Helminthosporium solani, Aspergillus niger, Penicillium digitatum and Mucor piriformis isolated from tomato. This research was undertaken to control the growth of these rot fungi in vitro. Extracts at various concentrations ranging from 0-30% were separately added to PDA media. The plates were inoculated separately with the fungal isolates. Effects of these extracts on mycelial growth of the fungi were highly significant ($P < 0.05$) for all treatments. Z. officinale extract at 25% and A. melegueta at 30% concentration gave complete inhibition. Phytochemical analyses of extracts revealed the presence of tannins, phlobatannins, steroids, tarpenes, saponins, flavonoids and alkaloids. The presence of these compounds supports the use of the extracts as antimicrobial agents which can prolong the shelf–life of fresh tomato fruits.
Keywords: Antimicrobial properties, Extract, Phytochemicals, Aframomum melegueta, Zingiber officinale.

1. Introduction

Fruits may be infected by pathogens when green and small, and may not show any symptoms until they ripen. The pathogens can, however, infect tissues that are wounded and exposed without a protective surface. Mechanical injuries (e.g. cuts and punctures) that occur during harvest and handling are good sites for decay development on the fruit surface (Jerry et al., 2005). By contrast, internalized pathogens (those that have entered tissues beneath the fruit surface) cause lesions that begin inside the fruit. Fruits, due to their low pH value, high moisture content and nutrient composition are very susceptible to attacks by pathogenic fungi, which in addition to causing rots, may also make them unfit for human consumption due to mycotoxins produced (Stinson et al., 1981; Philips, 1984; Moss, 2002). Fungi are the most important and prevalent pathogens, infecting a wide range of host plants and causing destructive and economically important diseases of most fresh fruits and vegetables during storage and transportation (Sommer, 1985). The use of synthetic fungicides control approach has proved effective in the control of phyto diseases. Most synthetic fungicides used in controlling some of these phyto pathogens are costly and therefore are not economically viable. The excessive use and misuse of chemical fungicides have raised serious concern about health and environmental hazards, and increasingly strict maximum residue limits. These have significant draw-backs including increased cost, handling hazards, and concern about fungicide residues on food (Tzortzakis and Economakis, 2007). It is obvious that recently, there has been a considerable interest in extracts and essential oils from aromatic plants with antimicrobial activities for controlling pathogens and/or toxin producing microorganisms in foods (Soliman and Badeaa, 2002; Valero and Salmeron, 2003) The use of pesticides and fungicides of botanical origin has been pin-pointed by many researchers as an option to synthetic fungicides (Amadioha and Obi, 1999; Amadioha, 2000).

A sizeable portion of the world population living below poverty line in the developing and underdeveloped countries of Africa are suffering from health problems associated with consuming mycotoxin contaminated grains, cereals, fruits and vegetables (Majumder et al., 1997). Even though effective and efficient control of seed borne fungi can be achieved by the use of synthetic fungicides, the same cannot be applied to fruits and vegetables for reasons of fungitoxic effects (Dukic et al., 2004). Thus, there is a need to search for alternative measures of protecting fruits and vegetables for human consumption from fungal infection. Plant extracts of many higher plants have been reported to exhibit antifungal properties under laboratory trials (Kiran and Raveesha, 2006; Mohana and Raveesha, 2006; Okigbo and Ogbonnaya, 2006).

Members of the family Zingiberaceae are important in traditional medicine for the treatment of many diseases such as inflammation, morning sickness in pregnancy and many infective diseases. In vitro studies have shown that active constituents of ginger inhibit growth of fungi and bacteria. Extracts from the seeds of Aframomum melegueta have potent antiseptic, fungicidal and bactericidal properties, and have, therefore, been used in preventions of infections and treating wounds (Okwu, 2004). The essential oil of Aframomum melegueta

has exhibited activity against gram positive and gram negative bacteria as well as Candida albicans. The extract of the rhizome of Z. officinale has pronounced inhibitory activities against Candida albicans that causes candidiasis (Gugnani and Ezenwanze, 1985; Mascolo et al., 1998; Deboer et al., 2005). Ginger extract containing gingerol inhibits the growth of many bacteria and fungi in vitro (Fricker et al., 2003).

Leaf extract of A. melegueta contains phytochemicals which offer an enormous potential as biocontrol agent of pathogens and a source of antimicrobial agents of therapeutic importance. The work of Doherty et al. (2010) revealed that its ethanolic extract has greater antimicrobial activity than its aqueous extract as indicated by the zones of inhibition. The efficacy of Azadirachta indica and Aframomum melegueta on fungi isolated from diseased cassava tubers was tested by Okigbo et al. (2009). These extracts which showed no significant difference in the yield of cassava were found to be fungitoxic to Fusarium oxalicum, Aspergillus niger and Botryodiplodia theobromae. Ethanolic extract of A. melegueta inhibited A. niger most. The major methods employed in prevention of the spread of these pathogens are application of fungicides, solarisation, and the use of antagonistic micro organisms among others. The use of synthetic fungicides is environmentally hazardous, and therefore, has been recently banned (Okereke and Wokocha, 2007). There is, therefore, the need to search for cheaper environmentally friendly and readily available alternatives such as plant extracts for the control of these pathogens. In view of the fore mentioned hazards the search for alternative means for combating fungal diseases in plants was embarked upon in this work. Hence the fungitoxic potentials of A. melegueta and Z. officinale were assessed and phytochemical analyses of their extracts ascertained.

2.0 Materials and Methods

2.1 Materials collection

The seeds of Alligator pepper, Aframomum melegueta and rhizomes of ginger, Zingiber officinale were purchased from Obudu market in Obudu Local Government Area of Cross River State. Fungal pathogens were isolated from diseased tomato fruits obtained from Onuiyi market in Nsukka, Nsukka Local Government Area of Enugu State. Identification was done in the department of Plant Science and Biotechnology, University of Nigeria, Nsukka.

2.2 Isolation and identification of isolates

The isolation technique used was based on (Chiejina, 2008) method. Thin sections (2mm diameter) were cut from the periphery of diseased tomato fruits and sterilized in 0.1% mercuric chloride for two minutes. The sections were rinsed in three changes of sterile distilled water and plated in Water Agar (WA) for 4 days. Fungal growth was transferred to Potato Dextrose Agar (PDA) plates. Culture plates were incubated at room temperature (27 ± 2^0 C) for 4-7 days. Several transfers of fungal growth were aseptically made from the PDA plates into clean PDA plates until pure cultures of the isolates were obtained. Identification was based on observations of culture growth patterns, colour of mycelia and microscopic examinations of vegetative and reproductive structures. Manuals and books (Barnett and Hunter, 1999; Alexopoulos et al., 2002) were used for the identification.

2.3 Preparation of samples

Four hundred grammes each of Aframomum melegueta seeds and Zingiber officinale rhizomes were dried at room temperature for three weeks. Two hundred grammes of the samples were ground separately and put into containers, labelled and stored for the extraction process.

2.4 Extraction procedure

The extraction process used was the soaking method (Doherty et al., 2010). One hundred grammes of each powdered sample was soaked in 1000ml of 70% methanol for 24 hours. The samples were each filtered twice through cheese cloth and collected in a round bottom flask. They were later concentrated using a rotary evaporator.

2.5 Antimicrobial properties of the extracts on the fungal isolates

Varying concentrations of the extracts 0%, 5%, 10%, 15%, 20%, 25% and 30% of A. melegueta and Z. officinale were separately incorporated into PDA plates. The plates were inoculated with the isolates (three replicates per pathogen for each concentration of the extracts) and incubated at room temperature. Diameter of mycelial growth was measured from two axis at 2 days intervals for 8 days.

2.6 Phytochemical analysis

Qualitative analyses of the constituents of the plant extracts were carried out.

2.7 Test for tannins

Two millilitres each of the methanolic extracts were separately boiled for ten minutes in 10ml of water in a test tube. A few drops of 0.1% ferric chloride were added to each test tube and observed for 10minutes for a brownish green or a blue black coloration (Okwu, 2005). This test was repeated once to confirm the results.

2.8 Test for phlobatannin

Two millilitres each of the methanolic extracts of the plant samples were boiled for 10minutes with 1% aqueous

hydrochloric acid. The deposition of a red precipitate was taken as evidence for the presence of phlobatannins (Okwu, 2005). The test was repeated once as above to confirm results.

2.9 Test for saponins

2.9.1Frothing test:

About 5ml each of the methanolic extracts of the samples were shaken with equivalent amount of water in a test tube for 5minutes. This was boiled in the water bath for 5-10minutes. Frothing that persists on warming was taken as evidence of the presence of saponins (Trease and Evans, 1989). The procedures were repeated as well to confirm the results.

2.10 Test for flavonoids

About 5ml of dilute ammonia solution was added to 3ml each of the methanolic extracts, followed by the addition of concentrated tetraoxosulphate (VI), (H_2S0_4). A yellow coloration was taken as evidence for the presence of flavonoids (Okwu, 2005). This test was repeated again to confirm results.

2.11 Test for alkaloids

About 2ml each of the extracts were stirred with 5ml of 1% aqueous hydrochloric acid on a steam bath for 10minutes; 1ml of the extract was treated with a few drops of Mayer's reagent, precipitation with these reagents was seen as evidence for the presence of alkaloids. The method was repeated again to confirm the results (Sofowora, 1993).

2.12 Test for steroids

One millilitre each of the extracts was dissolved in 2ml of chloroform. A few drops of concentrated sulphuric acid were carefully added to form a lower layer. A reddish brown colour formed at the interphase indicates the presence of a steroid ring (Sofowora, 1993). The same procedures were repeated once to confirm the results.

2.13 Test for terpenes

One millilitre each of the methanolic extracts were added to 2ml of chloroform and treated with five drops of acetic anhydride along with 2 drops of concentrated sulphuric acid. A pink colour formed at the interphase indicated a positive test of terpenes (Sofowora, 1993). This test was repeated once to confirm results.

2.14 Data analysis

Data were subjected to one way Analysis of variance (ANOVA) using Genstat statistical package. Means significant were separated by Fisher's Least Significant Difference (LSD) at P = 0.05 level.

3.0 Results and Discussion

The isolates were identified as Aspergillus niger, Penicillium digitatum, Helminthosporium solani and Mucor piriformis. The effects of A. melegueta and Z.officinale extracts at various concentrations on the growth of pathogens are shown in Tables 1 and 2 respectively. The results showed that the two extracts significantly ($p< 0.05$) reduced the radial growth of the pathogens with Z. officinale giving complete reduction of all the pathogens at 25% and above concentration (Table 2). At 30% concentration, both extracts completely reduced the mycelial growth of the pathogens. It was observed that the antifungal effectiveness of these extracts in culture is concentration dependent. This is in agreement with the works of Trease and Evans (1989); Amadioha and Obi, (1999) and Udo et al. (2001) who reported the high potency of plant extracts for the control of pathogenic fungi of other crops. The degree of control by these extracts varied and was highly significant at 25 and 30% respectively. Pre and post harvest bio-deterioration and spoilage of vegetables, fruits and agricultural produces due to infestation by microorganisms may cause losses of up to 100%. Association of variety of fungi including species of Aspergillus, Penicillium, Mucor and Helminthosporum causing significant loss in fruits and vegetables nutritional quality have been reported (Koirala et al., 2005).

Results of the phytochemical analysis revealed the presence of Tannins, phlobatannins, steroids, terpenes Saponins, flavonoids and alkaloids in both Aframomum melegueta seeds extract and Zingiber officinale extracts (Table 3). The presence of these phenolic compounds in these extracts indicates that these plants can serve as antimicrobial agents. This is because phenol and phenolic compounds have been extensively used in disinfection and remain the standard with which other fungicides are compared (Doherty et al., 2010). Phenolic compounds act as electron donors and are readily oxidized to phenolate ion or quinine, an electron acceptor (Doherty et al., 2010). The antifungal activity of the oil is believed to be associated with the phytochemical components of these plants (Matasyoh et al., 2007) which diffuse into and damage cell membrane structures.

Velluti et al. (2004) highlighted that generally, one of the critical things to consider for commercial applications is that the levels of essential oils and their compounds necessary to inhibit the microbial growth were higher in foods than in culture media. This is due to interactions between the phenolic compounds and the food matrix (Nuchas and Tassou, 2000). Extracts from seeds of A. melegueta and rhizomes of Z. officinale therefore have potent antiseptic, bactericidal and fungicidal properties. These findings support the use of these extracts for prevention of infections as also reported by Okwu (2004).

Results of this work suggest that fungitoxic compounds are present in Z. officinale and A. melegueta

extracts since they were able to control the growth of the fungal pathogens tested. This is in agreement with the work of Udo et al. (2001) who worked on the inhibition of growth and sporulation of fungal pathogens in Ipomoea batatas and Dioscorea sp by garlic extracts. The antimicrobial activity of these plants also agrees with the work of Adejumo and Langenkamper (2012), which showed that methanolic extracts of leaves of botanicals possessed antimicrobial properties. Also Okigbo amd Nmeka (2005) used leaf extracts of Xylopia aethiopica and Z. officinale to control yam tuber rot caused by Aspergillus niger, Aspergillus flavus and Fusarium oxysporum. A. melegueta extract was also used by Okigbo and Ogbonnaya (2006) in the control of F. oxysporum and A. niger rot in yam tubers.

Also the effect of aqueous extract of ginger was evaluated by Stangarlin et al. (2011) at the concentrations of 1, 5, 10, 15, 20 and 25% on Sclerotina sclerotiorum mycelial growth and sclerotia production, in vitro. The efficiency of protection of Z. officinale was also verified in lettuce plants. Besides the reduction in disease incidence, the authors reported that, the crop yield and the peroxidase induction were also analyzed in the plant tissues. This antimicrobial property of ginger in reducing the mycelial growth of fungal pathogens is in line with the results of this study. Ijato (2011) reported that extracts of Z. officinale and Ocimum gratissimum were mycotoxic to Fusarium oxysporum, Botrydioploida theobromae, Aspergillus flavu and Aspergillus niger of post harvest rot of yam tubers and that the effectiveness of the extracts increased with increase in concentration as was observed in this study (Tables 1and 2). Further studies on these effective botanicals should gear towards fractionation of the extracts which will lead to the isolation of the compounds that is showing considerable antifungal activity. The continuation of study on the plant is essential to isolate, identify, characterize and elucidate the structure of the bioactive compounds responsible for the observed antifungal activities. From this result, it is essential to investigate the specific constituents which are responsible for this observed activity. The in vivo study is also required to confirm the usefulness of the obtained results.

Conclusion

In conclusion, the in vitro results of this study confirmed the potentiality of A. melegueta and Z. officinale as one of the best sources for controlling fungal growth. Hence, further work is necessary to evaluate its potentiality in vivo on rot fungi and other pathogens. This can provide an alternative means for the control of tomato fruit rot by farmers. Investigations are also needed to characterize, formulate and market the active principles of these extracts which may provide avenues for the discovery of novel antimycotic compounds. These biofungicidal botanicals are environmentally safe; therefore, they could successfully replace the toxic and hazardous synthetic compounds and be exploited as ideal treatment for future plant disease management programs.

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References

Adejumo, T. O and Langenkämper, G. (2012). Evaluation of botanicals as biopesticides on the growth of fusarium verticillioides causing rot diseases and fumonisin production of maize. Journal of Microbiology and Antimicrobials, 4(1):23-31

Alexopoulos, C.O., Mims, C.W. and Blackwell, M. (2002). Introductory Mycology. 4th ed. John Wiley and Sons Inc. Singapore. 869pp.

Amadioha, A.C (2000). Fungitoxic effects of some leaf extracts against Rhizopus oryzae causing tuber rot of potato. Archives of Phytopathology P. Flan. 1:1-9.

Amadioha, A.C and Obi, V.I (1999). Control of Anthranose diseases of Cowpea by Cymbopogon cunitus and Ocimum gratissimum. Acto Phytopathology and Entomology 85: 89.

Barnett, H.L. and Hunter, B.B. (1999). Illustrated Genera of Imperfect Fungi, 4th ed. The American Phytopathological Society . St. Paul, Minnessota,USA, 218pp.

Chiejina, N.V (2008). Mycoflora of some salad vegetables. Bio-Research.6:392-395.

Deboer, H.J., Kool, A., Broberg, A., Mziray, W.R., Hedberg, I. and Levenfors, J.J. (2005). Antifungal activity of some herbal remedies from Tanzanias. Journal of Ethnopharmacology. 96:461-469.

Doherty, V.F., Olaniran, O.O. and Kanife, U.C. (2010). Antimicrobial activities of Aframomum melegueta (Alligator pepper). International Journal of Biology. 2(2):126-131.

Dukic, N.M., Bozin, B., Sokovic, M., Simin, N. (2004). Antimicrobial and antioxidant activity of Melissa officinalis L.(Lamiaceae) essential oil. Journal of Agricultural Food
Chemistry 52: 2485 – 2489.

Fricker, C.E., Smith, M.L. and Akpagana, K. (2003). Bioassay-guided isolation and identification of antifungal compounds from ginger. Journal of Phytotherapy Research. 17:897-903.
Guagnani, H.C. and Ezenwanze, E.C. (1985). Antibacterial activity of extracts of ginger (Zingiber officinale) and African oil bean seed (Pentaclethora macrophylla). Journal cummun Disease.17:233.
Ijato, J.Y. (2011). Inhibitory effect of two indigenous plant extracts of Zingiber officinale and Ocimum gratissimum on post harvest yam (Dioscorea rotundata) rot in vitro. Journal of American Science, 7(1):43-47.
Jerry, A.B., Steven, A.S and Michael, M. (2005). Guide to Identifying and controlling of postharvest Tomato diseases in Florida. Department of Horticultural Sciences, University of Florida/ IFAS, Gainesville. 131pp.
Kiran, B. and Raveesha, K.A., (2006). Antifungal activity of seed extract of Psoralea corylifolia L. Plant Disease Research 20: 213-215.
Koirala, P., Kumar, S., Yadar, B.K. and Premarajan, K.C. (2005) Occurrence of Aflatoxin in some of the food and feed in Nepal. Indian Journal of Medical Sciences 59: 331-336.
Majumder, U.K., Gupta, M. and Mukhopadhyay, D.K. (1997). Effect of mycotoxins isolated from Penicillium nigricans on glucose-6-phosphate dehydrogenase. Indian Journal of Experimental Biology 35:1233 – 1236.
Mascolo, N., Jain, R. and Jain, S.C. (1998). Ethnopharmacologic investigation of ginger (Zingiber officinale). Journal of Ethnopharmacology.27:129-140.
Matasyoh, J.C., Wagara, I.N., Nakavuma, J.L and Kiburai, A.M (2007). Chemical composition of Cymbopogon citratus essential oil and its effect on mycotoxigenic Aspergillus species. African Journal of Food Science, 5(3): 138-142.
Mohana, D.C. and Raveesha, K.A. (2006). Anti-bacterial activity of Caesalpinia coriaria (Jacq.) Willd. against plant pathogenic Xanthomonas pathovars: an eco-friendly approach. Journal of Agricultural Technology 2: 317-327.
Moss, M.O. (2002). Mycotoxin Review. 1. Aspergillus penillium. Mycologist. 16:116-119.
Nuchas, G.E and Tassou, C.C (2000). Traditional preservatives-oils and spices. In Robinson RK, Batt CA, Patel PD (Eds.), Encyclopedia of food microbiology. London, UK: Academic Press, pp. 1717−1722.
Okereke, V.C and Wokocha, R.C (2007). In vitro growth of four isolates of Sclerotium rolfsii, Sacc. in the humid tropics. African Journal of Biotechnology, 6:1879 – 1881.
Okigbo, R. N. and Nmeka, I. A. (2005). Control of Yam tuber rot with leaf extracts of Xylopia aethiopica and Zingiber officinale. Africa Journal of Biotechnology. 4(8): 804-807.
Okigbo, R.N and Ogbonnanya,O.U (2006). Antifungal effects of two tropical plant extracts Ocimum gratissimum and Aframomum melegueta on post harvest yam Dioscore spp rot. African Journal of Biotechnology, 5:727-731.
Okigbo, R.N., Ramesh, P. and Achusi, C.T (2009). Post-Harvest Deterioration of Cassava and its Control using extracts of Azadirachta indica and Aframomum melegueta. E-Journal of Chemistry. 6(4):1274-1280
Okwu, D.E. (2004). Phytochemicals vitamins content of Indigenous spices of South Eastern Nigeria. Journal of Sustain Agricultural Environment.6:30-34.
Okwu, D.E. (2005). Phytochemicals, vitamins and mineral contents of two Nigeria medicinal plants. International Journal of Molecular Medicine and Advance Sciences, 4:375-381.
Phillips, D.J. (1984). Mycotoxins as a post-harvest problem, pp50-54. In: Post-harvest Pathology of Fruits and Vegetables; Post-harvest losses in Perishable Crops. Moline, H.E. (ed). Agricultural experimental station. University of California, Berkeley Publications. NE, 461pp.
Sofowora, A. (1993). Medicinal plants and Traditional medicine in Africa. Spectrum Books limited, Ibadan, Nigeria, 346pp.
Sommer, N.F. (1985). Strategies for control of post-harvest disease of selected Commodities, pp83-98. In: Post-harvest Technology of Horticultural Crops. University of California Press, 246pp.
Stangarlin, J.A., Kuhn, O.J., Assi, L. and Schwan-Estrada, K.R.F (2011). Control of plant diseases using extracts from medicinal plants and fungi, 1033-1042pp. In: Communicating Current Research and Technological Advances. (Mendez-Vilas Ed).
Stinson, E.E, Osman, S.F., Heisler, E.G., Sicihano, J. and Bill, D.D. (1981). Mycotoxin production in whole tomatoes, apples oranges and lemons. Journal of Agriculture and Food Chemistry.29:790-792.
Trease, G.E. and Evans, W.C (1989). Pharmacognosy, 13th ed. Balliere Tindall, London, 546pp.
Udo, S.E., Madunagu, B.E and Isemin, C.D (2001). Inhibition of growth and sporulation of fungal pathogens on sweet potato and yam by garlic extract. Nigerian Journal of Botany 14:35-39.
Velluti A, Marlın S, Gonzalez P, Ramos AJ, Sanchis V (2004). Initial screening for inhibitory activity of essential oils on growth of Fusarium verticillioides, F. proliferatum and F. graminearum on maize-based agar media. Food Microbiology,21: 649–656.

Table 1: Effect of *Aframomum melegueta* extracts on the mycelial growth of the pathogens.

Fungi	Mycelial radial growth (mm)						
	0%	**5%**	**10%**	**15%**	**20%**	**25%**	**30%**
A.niger	*90.00a	74.67a	60.67a	44.33a	28.33a	9.67a	0.00a
H.solani	88.33b	70.33b	52.00b	34.67b	16.33b	0.00c	0.00a
M.piriformis	90.00a	73.33c	52.67b	35.00c	16.67b	0.00c	0.00a
P.digitatum	88.00b	72.33c	56.00c	38.88d	22.67c	7.33b	0.00a

LSD (P<0.05) = 1.0952
*Each of the data is a mean of three replicates. Mean values in a column with the same alphabets are not significantly different at P < 0.05.

Table 2: Effect of *Zingiber officinale* extracts on the mycelial growth of the pathogens.

Fungi	Mycelial radial growth (mm)						
	0%	**5%**	**10%**	**15%**	**20%**	**25%**	**30%**
A.niger	*90.00a	63.33a	46.67a	28.00a	16.33a	0.00a	0.00a
H.solani	88.33b	64.00a	45.33b	26.00b	14.33b	0.00a	0.00a
M.piriformis	90.00a	66.33b	48.67c	25.67b	12.67c	0.00a	0.00a
P.digitatum	88.00b	67.00b	50.00d	26.33b	15.33ab	0.00a	0.00a

LSD (P<0.05) = 1.0952
* Each of the data is a mean of three replicates. Mean values in a column with the same alphabets are not significantly different at P < 0.05.

Table 3: Phytochemical screening of the *A. melegueta* and *Z. officinale* extracts

Compounds	***A. Melegueta***	***Z. officinale***
Tannins	++	++
Phlobatannins	++	+
Steroids	++	++
Terpenes	++	+
Saponins	+	+
Flavonoids	+	+
Alkaloids	+	++

++ = Present in high amount
+ = Present in moderate amount