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Antifungal Efficacy of Allium Sativum and Zingiber Officinale on Fungi Isolated from Spoilt Bread

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Abstract: *Zingiber officinale* is a common condiment for various foods and beverages and a history of important traditional medicine herb for the treatment of stomach disorder. This study deals with the antifungal activity of *Allium sativum* and *Zingiber officinale* and their phytochemical composition. Ethanolic extracts of two spices *(Allium sativum and Zingiber officinale)* were tested for antifungal activities against *Aspergillus* sp, *Penicillium* sp and *Fusarium* sp using mycelia growth extension method, result showed varying degrees of antifungal activities against the test fungi. The two extracts showed similar pattern of antifungal activities on the test fungi withthe extract of *Allium sativum* being more effective with increased zones of inhibition with increased concentrations. Phytochemical screening revealed the presence of flavonoids, saponins, terpenoids and tannin for *Allium sativum* and flavonoids, glycosides, tannins and anthraquinone for *Zingiber officinale*. The significant growth of inhibitions of the test fungi by the plant extract suggest the possible use of these spices in controlling infections caused by these fungi and food spoilage.

Keywords**:** *Allium sativum, Zingiber officinale,* Ethanolic extract, Phytochemical screening.

1. INTRODUCTION

Historically, medicinal plants have been a source of novel drug compounds, plants derived products have made large contributions to human health and wellbeing.

Over the years, much efforts have been devoted tothe search for new antimicrobial agents from natural sources such as plants and others for treatment and for food preservations. Many scientists across the globe have reported antimicrobial properties of several medicinal plants but still a very meager portion of this tremendous potential drug-repertoire has been scientifically screened ().

Spices and herbs have been used as food additives since ancient time, as flavouring agents and also as natural food preservatives (Mariya *et al*., 2009). They serve as item of international commerce for many hundred years. Several spice extracts have broad spectrum antimicrobial properties and can be recognized as bio-preservatives, having no harmful effect on human health (Akponah *et al.*, 2013). Many of these spice extracts possess significant antimicrobial activity which in many cases is due primarily to particular constituent such as alkaloid phenols, glycosides, steroids, tannins or essential oils (Lopez et al., 2006). A number of spices show antimicrobial activity against different types of microorganisms and these activities depend on the microorganism, the type of specie, the test method as well as the chemical composition and content of extracts and essential oils (Laura, 2007). Among these specie which are available and cheaper than the conventional drugs include garlic and ginger which in their natural states are widely used in West Africa as herbal medicine in the nineteenth century, ginger served as a popular remedy for cough and asthma when the juice is mixed with little juice of fresh garlic and honey (Foster, 2000).

Zingiber officinale belongs to the family *Zingiberaceae* while *Allium sativum* on the other hand belongs to the family *Alluaceae*, they have been used for decades in treating cold induced diseases such as nausea, asthma, cough, hearth palpitation, swelling, dyspepsia, loss of appetite and rheumatism (Foster, 2000).

The anti-inflammatory properties of ginger and garlic have been known and valued for centuries. The original discovery of ginger's inhibitory effects on prostaglandin biosynthesis in the early 1970s has been repeatedly confirmed. This discovery identified ginger as a herbal medicine product that shares pharmacological properties with non-steroidal anti-inflammatory drugs. Ginger is a strong anti-oxidant substance and may either mitigate or prevent generation of free radicals. It is considered a safe herbal medicine with only few and insignificant adverse/side effect.

The approved modern the rapeutic applications for ginger are supportable based on its history of use in an established systems of traditional and conventional medicine, extensive phytochemical investigation, pharmacological studies in animals and human clinical studies.

This study was aimed at evaluating the Antifungal potential of *Zingiber officinale* and *Alliumsativum* against *Penicillium* species, *Fusarium* species and *Aspergillus* species.

2. MATERIALS AND METHODS

This research was carried out in the Department of Applied Science, Microbiology Laboratory of Kaduna Polytechnic.

Collection and Handling of Plant Materials

The *Allium sativum* and *Zingiber officinale* were purchased in Central Market in Kaduna State, Nigeria. The authentication of the species was done by comparing them with the voucher specimens available in Department of Biological Science Faculty of Life Science, Ahmadu Bello University, Zaria, in an air tight container. The samples were conveyed to the Microbiology Laboratory Department of Applied Science, Kaduna Polytechnic until required.

Plant Extraction

The *Allium sativum* bulbs and *Zingiber officinale* rhizomes were separately washed with distilled water and aseptically peeled with a sterile knife. They were crushed separately using sterile mortar and pestle, and transferred into two stoppered vessels. Five hundred (500ml) of ethanol was poured into each of the vessels containing the crushed sample and the system were allowed to stand for a day without shaking after which the system was subjected to occasional shaking for another six days. The liquids were then strained off and filtered using Whatman no 1 filter paper. The recovered filtrates were evaporated using water bath and resultant crude extracts were used for antifungal activity testing.

Isolation of Test Organisms

The Aspergillus, Penicillium and the Fusarium species were isolated from spoilt bread using Sabouraud's Dextrose Agar. Different colours were observed on the spoilt bread as a result of different fungal growth,they were inoculated on petri dishes containing solidified Sabouraud Dextrose Agar. They were incubated at room temperature for 5 days, the resultant mixed cultures were subcultured so as to obtain pure cultures.

Macroscopic Identification of the Fungal Isolates

The fungal isolates were identified macroscopically using cultural appearance and cultural characteristics with the aid of a hand lens. Different colours such as black, green, pink and white were seen on the plates.

Microscopic Identification Using Lactophenol

The Smears from the culture were prepared and stained with lactophenol. The preparations were observed under x10 and x40 objectives of the microscope and compared with the fungi identification key for inference.

Phytochemical Screening of the Extracts

Phytochemical screening was carried out on the two extracts using the standard methods.

Test for Saponin

Exactly 5g each of the two extracts were accurately measure and transferred into 5ml distilled water to two separate test tubes. A frothing appearance on shaking with water indicates the presence of saponin.

Test for Tannins

Five (5ml) each of the two extracts were accurately measured and transferred into 10ml distilled water in two separate test tubes; ferric chloride reagent was added. Blue black or blue green precipitate appearance indicates the presence of tannin.

Test for Flavonoids.

In order to test flavonoids, 5ml each diluted ammonia solution was added into 5ml each of the extract in separate test tubes. 3ml of concentrate $H_2S0_4$was added, a yellow coloration indicates the presence of flavonoids.

Test for Terpenoids

Two grams (2ml) each of chloroform was added into 1ml of the extracts in separate test tubes and 3ml of concentrated $H_2S0_4$was added, a reddish-brown coloration at the interface indicate the presence of terpenoids.

Test of Steroids

Two (2ml) each of acetic anhydride was added to 3ml each of the two extracts in a separate test tubes and 2ml of sulphuric acid was added by the sides of the test tubes. A color change of violet or blue indicated the presence of steroids.

Test for Anthraquinone

Exactly 5g/ml each of the two extracts were taken into a dry test tube and 5ml of chloroform was added shaken for 5 minutes. Equal volume of 10% ammonia solution was added into the test tube. A pink violet or red colour in the ammonical layer (lower layer) indicates the presence of anthraquinone.

Test for Glycosides

Five (5ml) each of the two extracts were dissolved in 1ml of glacial acetic containing one drop of ferric chloride solution. 1ml of concentrated sulphuric acid was added into the test tube. A brown ring obtained at the interface indicates the presence of glycosides.

Determination of Plant Yield

The percentage yield was obtained using the formula $^{w1}/_{w2}$ x $\frac{100}{1}$. Where$w1$ is the initial is weight of the sample before extraction and $w2$is the final weight of the sample afterextraction.

Antifungal Testing

The effect of the spice extracts was determined by measuring the mycelial extension of the fungi on Sabouraud's Agar medium, 2ml each of the spice extract was mixed with 200ml of sterile Sabouraud's Dextrose Agar medium in test tubes and poured into sterile petri dishes. Streptomycin, 0.5mg/litre was prepared and was added to the mixture of the medium and the spices to suppress the growth bacteria. The plants were allowed to cool and solidified while the test organisms were inoculated into the medium. Mycelial disc (5mm in diameter) was collected on the surface of actively growing fungal culture using a sterile cork borer and was placed at the central of the medium containing the extract. Two plates were prepared for each spice and for each fungus under aseptic conditions. The control plates without the spice extracts were similar inoculated with the fungi. The set up were incubated at room temperature for five days in the laboratory. The radial growth less the diameter of the initial inocula was measured in two directions along the perpendicular lines and the means were calculated for each plate and with the fungal growth.

Preparation of Varied Concentrations of the Extract

Different concentrations of the two extracts were prepared using distilled water. Exactly 0.1g each of the extracts were dissolved in 10ml each of the distilled water in a test tube to obtain the 100mg concentration. Exactly 5ml was taken from the 100mg concentration and dispensed into 5ml of distilled water to get the 50ml/ml concentration. Another 5ml was also taken from the 40mg concentration and dispensed into 5ml of distilled water to obtain the 25mg concentration and finally 5ml was taken from the 25mg concentration and dispensed into 5ml of distilled water to obtain the 12.5 concentration. Four different concentrations (100mg, 59mg, 25mg and 11.5g) were prepare for each of the extracts and these varied concentration were used against the isolates to determine the Minimum Inhibitory Concentration (MIC) (Cheesbrough, 1994).

Determination of Minimum Inhibitory Concentration of Spice Extract

Exactly 20ml of Sabouraud's Dextroxe Agar was poured into sterile petri dishes containing different concentration (100mg, 50mg and 12mg) of the respective extracts of the species (Ginger and garlic) Mycelial discs (5mm in diameter) was collected from the surface of actively growing fungal culture using a sterile cork borer and was placed at the centre of the Sabouraud's Dextrose Agar medium containing the extract. The Minimum inhibitory Concentration (MIC) was determined as the least concentration of spice extract that showed an inhibitory effect in the mycelial growth of the test fungi when compared with the control using the radial growth method (Cheesbrough, 1994).

Determination of the Minimum Fungicidal Concentration of the Extracts

The *in vitro*fungicidal activities were determined for each plant extract. After 72 hours of incubation, 1ml was subcultured from each culture that showed complete inhibition (100% or an optically clear plate), from the last positive culture (growth similar to the growth on the control culture and from the growth control (Extract free medium) on to Sabouraud's Dextrose Agar plates and were incubated at room temperature until growth was seen before 48hours. The Minimum Fungicidal Concentration (MFC) was determined as the lowest concentration that showed no growth of fewer than 3 colonies.

3 RESULTS AND DISCUSSIONS

RESULT AND DISCUSSION

Table 1: Percentage Yield of the Spice Extracts in Ethanol

Extract	**Weight 1 (w1)**	**Weight 2 (w2)**	**Yield &**
Allium Sativum	20kg	15kg	13.33%
Zingerber officinale	20kg	13kg	15.38%

Table 2: Phytochemical properties of *Allium sativum* and *Zingiber officinale* Extracts.

Extracts	Phytochemical screening						
	Flavonoid	Terpenoids	Saponins	Glycoside	Glycoside Tannins	Steroids	Anthraquinone
Allium sativum	+	+	+	-	+	-	+
Zingiber offic inal e	+	-	+	+	+	-	_

Table 3: Antifungal activity of Allium sativum and Zingiber officinale extract (diameter of mycelia extension) (mm)

Extract	Test Organism		
	Penicillium	Aspergillus	Fusarium
Allium sativum	25	17	5
Zingiber officinale	25.5	19	8.5

Table 4: Minimum Inhibitory Minimum Fungicidal Concentration of Allium Sativum (mg/ml)

Organisms	Concentration				
	0	12.5	25	50	100
Penicillium Specie	+	+			-
Aspergillus Specie	+	+	+	+	-
Fusarium Specie	+	+	+	-	-

Table 5: Minimum Inhibitory Minimum Fungicidal Concentration of Zingiber officinale Extract (mg/ml)

Organisms	Concentration				
	0	12.5	25	50	100
Penicillium Specie (mm)	+	+	+	-	-
Aspergilles Specie	+	+	+	+	-
Fusarium Specie	+	+	-	-	-

The percentage yield of the extract of *Alliumsativum* was found to be 13.33% while that of *Zingiberofficinale* was 15.38%. However the result in table 1 showed a wide range of yield among the two extract with *Alliumsativum*having a lower percentage yield and Zingiber officinale revealed the presence of some bioactive compounds which are responsible for their antifungal activities. This result is similar to the report of Roy et al., (2006) who observed that the phytochemical constituents of *Allium sativum* are responsible for

its antifungal properties. The antifungal evaluation of ethanolic extracts of *Allium sativum*and Zingiber officinale revealed a significant antifungal potency against the test organisms. Varying degrees of antifungal activities by the two extracts were observed at different concentrations, the spices showed antifungal activity against the fungi tested. *Fusarium*specie was observed as the most susceptible fungus while *Penicillium*specie was the least susceptible

fungus to the crude extracts of both spices as shown in table 3. Results showed that the two spices have similar antifungus activates the test fungi (Table 3). Both spices were also observed to have similar Minimum Inhibitory Concentration against *Fusarium*specie at 50mg/ml compared with *Zingiberofficinale*which have Minimum Inhibitory Concentration at 100mg/ml against *Fusarium* species. The strong inhibition potential of *Zingiberofficinale*is attributed to the fact that it contains over 400 different compounds, a mixture of both volatile and non-volatile chemical constituents such as Zingerons, shigaols, gingerols, sesquiterpenoids and a small monoterpenoid fraction (Chrebasik et al., 2005). The antifungal activity of garlic extract is found to be very effective in inhibiting the growth of *Aspergillums* species (Dankert et al., 2009)

The antimicrobial activity of extracts of Allium sativum have lobe been linked to the presence of some bioactive compounds, these secondary metabolite also serve to protect the plants themselves against bacterial fungal and viral infections (EL-Mahmood and Amey, 2007). These bioactive compounds are known to work synergistically to produce various effects on the human and animal subjects (Amagase, 2006). The antifungal potency of these spices also supported the work of Udo et al., (2001) who reported that methanol and ethanol extracts of*Alliumsativum*have high potency for the control of pathogenic fungi in potato and yam and tubers. In this study, it was observed that the higher of concentration of the ethanolic extracts of the spices, the higher the potency in the inhibition of mycelial growth of the fungi tested (Table 4). The Minimum Fungicidal Concentration against the test isolates was established for the two extracts in table 4.5. The two extracts showed fungicidal activities against the test isolates at 100mg/ml concentration except of *Alliumsativum*which showed a fungicidal activity on *Fusarium* specie at 50mg/ml (Banso et al., 1999). Reported that the antifungal substances contain in the extracts were fungicidal at higher concentrations. This study indicate the Minimum Fungicidal Concentration than in the Minimum inhibitory concentration assays but not at lower concentration as shown in Table 5.

4.0 CONCLUSION

This study has demonstrated the effectiveness of *Alliums ativum*and *Zingiber officinale* extracts against *Aspergillus* species, *Penicillium* species and *Fusarium*species. This has provided justification that if well processed, *Alliums ativum* and *Zingiberofficinale*can be used to develop bioactive substances that may have promising effect on the treatment of some infections. The extracts exhibit fungicidal properties that support their traditional use as antimicrobial (Cowan, 1999). *Allium sativum* and *Zingiber officinale* therefore have great potentials for the development of antimicrobial drugs most especially for the treatment of fungal infections.

RECOMMENDATIONS

Based on the major findings of the study, the following recommendations were made;

1. Further work on the phytoconstituents, isolation, purification and characterization of the bioactive components of these plants is recommended as it could lead to the development of more effective substances that can be used to treat infections.

2. It has been found in this study that *Alliumsativum* and *Zingiberofficinale* extracts possess antifungal potency, it is more therefore imperative that phymaceutical companies should explore them as a source of antifungal drugs.

3. *Alliumsativum* and *Zingiberofficinale* should be included as constituents in drugs formulation.

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