ISSN No:-2456-2165

Antifungal Effectiveness of *Zingiber Officinale* Plant Root Extract on *Aspergillus Niger* and *Rhizopus Stolonifer* Compared with Standard Drug

Abiodun Moses Oluwaseun[1], Abiodun Iyabode Khadijat[2], Kalu Mary Udochukwu[2]
[1]*Department of Biological Sciences, Faculty of Natural Sciences, Chukwuemeka Odumegu Ojukwu University, Uli, Anambra State, Nigeria*
[2]*Department of Microbiology/ Biochemistry, School of Industrial and Applied Sciences, Federal Polytechnic Nekede, Owerri, Imo State, Nigeria*

Abstract:- The effect of Zingiber officinale (Ginger) on two Fungi strains; Aspergilus niger and Rhizopus stolonifer was studied. The plant extracts were obtained by weighing 16g of powdered root of Zingiber officinale and dissolved in 800ml of acid water and distilled water respectively. The extracts were later subjected to gentle concentration using a hot water bath at 80^0C and then stored in different containers and refrigerated until the extraction was completed. Commercially prepared antiseptic Trichlorophenol (T.C.P) produced by Pfizer Pharmaceutical Company was employed as a standard drug for this study. Various dilutions of the plant extracts were prepared by mixing various volume of the extract and molting fungi growth media at ratio 1/2, 1/5, 1/10, 1/20, 1/50. The two fungi strains tested were freshly isolated from spoiled bread and it was identified in the department of Science Laboratory Technology of Osun State Polytechnic Iree Nigeria. Potatoes dextrose agar was Prepared by suspending 39grames of powdered (P.D.A) in 100ml of distilled water and boiled to dissolve completely,and sterilized with autoclave at 15bar at 121^0 c for 15min and various dishes were prepared.The inoculation of fungi was carried out using a standard procedure. All the plates were incubated at 37^0C for 24hrs, both the acid water and distilled water extract proved to be fungistatic on the two fungi strains tested and standard drug proved to be fungicidal. The (Maximum inhibitory concentration (MIC) of distilled water extract of the plant was observed at 1/30 dilution with (65.5%) mycelia growth inhibition on Aspergillus Rhizopus Stolonifer , while that of acid water extract was observed on Aspergillus niger at 1/10 dilution with (62.0%) mycelia growth inhibition and 1/5 dilution with (55%) mycelia growth inhibition on Rhizopus stolonifer .Based on the result of this study it shows that Zingiber officinale proved to be highly fungistatic when both distilled water and acid water were used as solvents for extraction when compared with the standard drug. There is need for Pharmaceutical companies in Nigeria to exploit the medicinal potential of this plant root in other to serve as source of raw material for the production of standard antifungal drugs.

Keywords:- *Zingiber officinale, Aspergillus niger, Rhizopus stolonifer , Trichlorophenol, Distilled water, Acid water, Antifungal drug.*

I. INTRODUCTION

Many intact leaves, steam and root produce antimicrobial substance that is probably responsible for the plant natural resistance to microbial infection (Oyewole, 2009). This quality of plant species have greatly utilized in traditional medicine, the Nigeria flora has always made and will continue to make a great contribution to the health care of Nigeria (Oliver, 2008). The herbalists employ two kind of treatments i.e. real and physiological the plant part commonly used include fruits, leaves, stem, root and bark different herbal medicine can be either raw or boiled concoction or de concoction, soup, ointments, lineament or incision is done with a sharp razor blade, the cut is rubbed with powder already prepared from herbs. These incisions are used to cure various diseases like headache, backache, and Rheumatism. Generally, a lot of work on antimicrobial activity of plant have been documented, in 1977, Tripath discovered a Fungi toxic principle from leaves of *Lawsonis inermis lam* on fungi on chemical analysis the antifungi factor was found to be 2-hydroxy 1-1-4 –napthaquinone (Lasone) . Lawsome was found to exhibit fungicidal activity, a wide variety of fungi toxic spectrum and non phototoxic compound completely inhibited the growth of *Helminthesporrum orize* (Ekundayo, 2006).

The root of many plant have a wide range of medicinal usage as chewing sticks and are pungent when chewed. *Zanthoxylum xanthoxyloides* lam has been reported to contain chemical with anaesthetic (pellitorine),anticancer (Fagaram),antisickling (O-hydroxy methyl betoic acid),antimicrobial (6-cathionine Chlerythrine berbine) properties have been identified from the root (Sofowora and Oyewole,1988. In 1996, (Egawa, *et al*., 1996) reported that antifungal substance was detected in leave of eleven out of Twenty seven species of Eckalyptus examined and that the antifungal substance consisted of 4 different component. Moreover, (Narian and Satapathy, 2014) demonstrated the antifungal activities of *Vicarose* extract of leaf, flower, Steam

ISSN No:-2456-2165

and root showing antifungal activites against *Helminthosporium rodulosim ,Scelerotium* rolfisic, Petalotia species, *Fusarium oxylosporium, Collectotricum speaces and Aspergillus niger* .While in 2008, Boake –yiaden reported that antibacterial activity of Bryophylum pinnatum juice against *Staphylococcus aureus* , *Bacillus subtilis* , *Escherichia coli*, while the oil of *Cymobpogon citrates* exhibited antimicrobial activity against 15 fungal species tested (Gbeile and Adesina, 1986). In 2003, Adesogan reported that *Diospyros Monbutulensis* with papery bark and yielding pulumbaginal antibiotic .The extract of which inhibited the growth of *Saccharomyces species* and *E.coli* and two other organisms. The plant like *Plumbago Zeylamica* can be other sources of plumbagin and it is derivatives (Narain, et al ., 2009).

Oloke and Kolawole , (2004) reported the antimicrobial and antifungal activity of certain components of *Aframomum maleguate* (Atare in Yoruba.)Fruit while (Farag, *et al*., 1989).Reported that essential oil of Thyme cumin clove completely inhibited both mycelia growth and afflatoxin production in *Aspergillus parasiticuse* in another related investigation, (Akinyanju, 2012) reported the activity of crude of aqueous extract of leave of *Acelyphatorta* against some microorganism while Okale,et al., (1990).Reported the trypanocidal activity of a aqueous extract of *Acehypha hispida* leaves (Okanla, *et al*., 1990). Olorundare, *et al*., 2016).Reported the antimicrobial activity of *Casialata* against gram positive *Staphylococcus faecalis* and *Micriococus luteous*. The major aim of this study is to determine Antifungal effect of *Zingeber officinale* (Ginger) on *Aspergilus niger* and *Rhizopous stoloniofer* and compared with standard antiseptic drug in other to provide a based line information and reference data about the potential antifungal properties of the plant which is a preliminary report towards antifungal drug development with *Zingiber officinale* root as a source of raw materials.

II. MATERIALS AND METHODS

➢ *Plant collection*

Zingiber officinale root was obtained from a local herb seller at Obada market in Iree, Osun State Nigeria and it was identified in the department of Science Laboratory Technology of Osun State Polytechnic and a plant voucher number was assigned to the plant as 24631.

➢ *Acid water preparation*

The acid water for this study was prepared by soaking maize seed in water for about two days until a PH of about 4.4 was obtained .The acid water was then filtered using a simple filtration technique.

➢ *Preparation of plant extract*

A healthy fresh plant root of *Zingiber officinale* was sundried for one week and it was blended into powdery form using blending machine ,mortar and pestle.16grams of powdered root was extracted with 800ml of acid water and distilled water respectively .The extraction was carried out for 72 hours using acid water and distilled water (Olorundare, *et al*., 1992).

The water extracts were later subjected to gentle concentration using a hot water bath at 80^0C; the extracts obtained were then stored in different container and kept in the refrigerator until extraction was completed.

➢ *Commercial Antiseptic*

The commercially prepared Trichlorophenol (T.P.C.) produced by Pfizer product limited Nigeria was employed in this study ,different dilution of the antimicrobial drug were Prepared in order to determined it antifungal effectiveness the various dilution used are 1/2 ,1/5, 1/10, 1/20, and 1/50.

➢ *Preparation of test organism*

The two fungal strains used are *Aspergillus niger* and *Rhizopous stolonifer* which was freshly isolated from spoilt bread and it was identified in the department of Science Laboratory Technology of Osun State Polytechnic Iree Nigeria.

➢ *Media Preparation*

The Potatoes Dextrose Agar was prepared by suspending 39grams of powdered (P.D.A) in 100ml of distilled water and boiled to dissolve completely; the medium was then sterilized using autoclave at 151bs pressure at 121^0 C for 15min. It was then mixed well before pour plating into a sterilized Petri dish, various concentrations of the extract and antiseptic were prepared by mixing known volume of the extract and antiseptic with P.D.A in a sterilized Petri dishes. Two drops of streptomycin sulphate was later added to each dish to inhibit the growth of bacteria contaminant. The control plates contained 10ml of the potatoes dextrose agar only.

Concentration	volume of molten agar (ml)	Extract content /ml
1/5	8.00	2.00
1/10	9.00	1.00
1/20	9.50	0.50
1/30	9.67	0.33
1/50	9.80	0.20

Table 1:- Acid water extracts plates

Concentration	Volume of growth media content (ml)	Extract content (ml)
1/5	8.00	2.00
1/10	9.00	1.00
1/20	9.50	0.50
1/30	9.67	0.33
1/50	9.80	0.20

Table 2:- Distilled water extract plates

ISSN No:-2456-2165

Concentration	Volume of molten growth media	Extract content (ml)
1/2	5.00	5.00
1/5	8.00	2.00
1/10	9.00	1.00
1/20	9.50	0.50
1/50	9.80	0.20

Table 3:- Standard antiseptic media plate

➢ *Inoculation of fungal strain*

The inoculation was carried out by using a sterile stainless cork borer to remove inoculum plug of 5mm diameter. From the advance edge of 48hours culture of *Aspergillus niger* and 18 hours *Rhizopus stolonifer*. The inoculum plug were transferred using sterile picker, all plates were incubated at room temperature.

➢ *Determination of mycelia growth inhibition*

The mycelia growth was measured at the end of the incubation for both organism .The diameters of the mycelia was measured along two perpendicular axis marked on the bottom of the plates, the average diameter was calculated and corrected for the 0.5cm of the inoculums plug. The measurements for each concentration of the antimicrobial agent and for the control were compared in other to determine the growth inhibition in percentage.

The mathematical representation of this as follows

$$\frac{P^1 - Z^1}{Z^1} \times 100/1 = Q\ \%$$

P^1 = Radial growth on control plate

Z^1 = Radial growth on antimicrobial agent

Q = % mycelia growth inhibition

III. RESULTS

After 24hours of inoculation of *Aspergillus niger and Rhizopus stolonifer* on different plates with different concentration of the extract in acid water, distilled water with the control plate and the commercial antiseptic plate, The percentage mycelia growth inhibition was calculated and the result was plotted on the graph against the time interval.

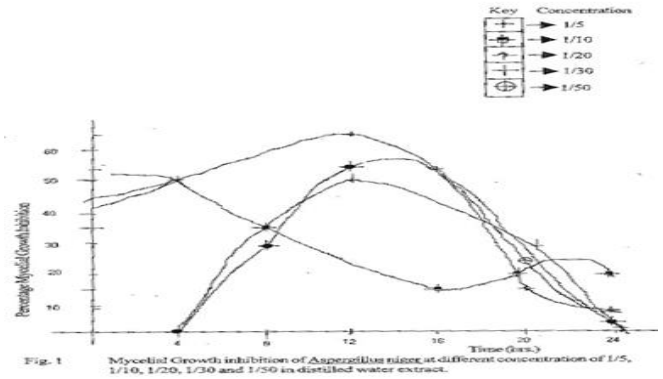

Fig. 1 Mycelial Growth inhibition of Aspergillus niger at different concentration of 1/5, 1/10, 1/20, 1/30 and 1/50 in distilled water extract.

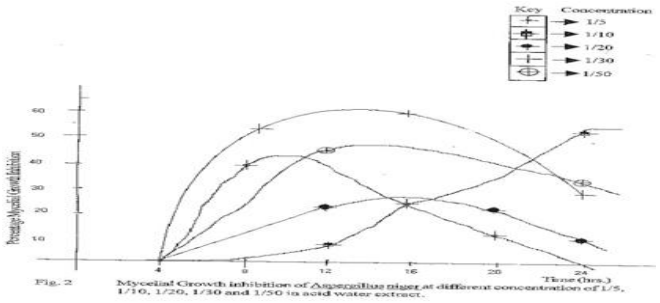

Fig. 2 Mycelial Growth inhibition of Aspergillus niger at different concentration of 1/5, 1/10, 1/20, 1/30 and 1/50 in acid water extract.

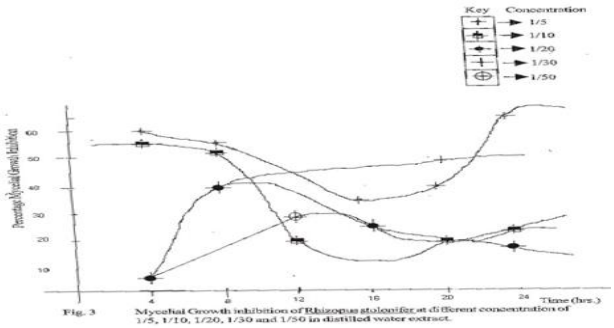

Fig. 3 Mycelial Growth inhibition of Rhizopus stolonifer at different concentration of 1/5, 1/10, 1/20, 1/30 and 1/50 in distilled water extract.

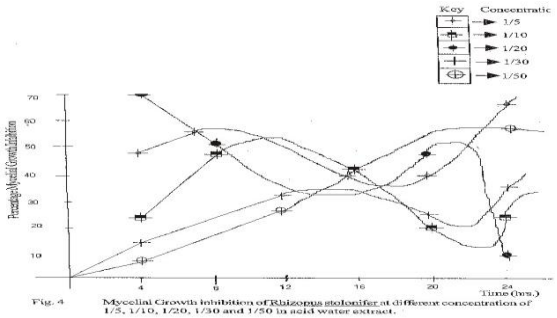

Fig. 4 Mycelial Growth inhibition of Rhizopus stolonifer at different concentration of 1/5, 1/10, 1/20, 1/30 and 1/50 in acid water extract.

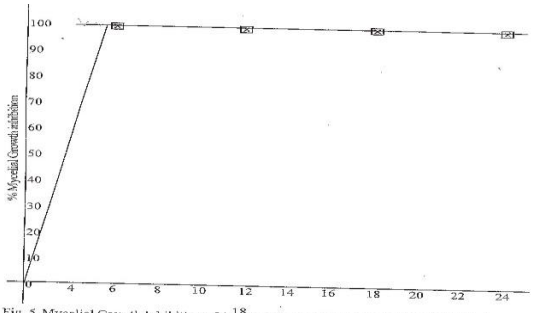

Fig. 5 Mycelial Growth Inhibition of Aspergillus niger in Trichlorophenol (TCP) at concentration 1/2, 1/5, 1/10, 1/20 and 1/50

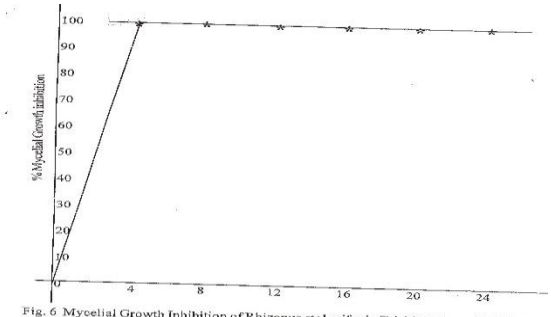

Fig. 6 Mycelial Growth Inhibition of Rhizopus stolonifer in Trichlorophenol (TCP) at concentration 1/2, 1/5, 1/10, 1/20 and 1/50

ISSN No:-2456-2165

IV. DISCUSSION

The antifungal effectiveness of the root extract of *Zingiber officinale* both in distilled water and Acid water was proved to be fungistatic on the *Aspergillus niger* and *Rhizopus stolonifer* while the standard Antiseptic Trichlorophenol have fungicidal activity on both tested fungi. It can be deduced from Fig 1, 2, 3, and 4 that the rate of inhibition of the two fungi varies directly with the concentration of the extract, indeed slightly increase or decrease the concentration of both extract dramatically increase or decrease the inhibitory activity of the extract and in context, the effect of dilution on the activity of the extract can be of crucial importance .Thus there is gradual increase in the inhibitory activity of the extract as the concentration of the extract increase in both the acid water and distilled water. More so, the percentage mycelia growth inhibition from Fig 1-4 shows that for each concentration of the extract, the inhibitory action of the acid water in all concentration used was greater than that of the distilled water extract on the fungi strains tested. The fungistactic action of the extract increase to a certain time and thereafter decrease. Fig 5 and 6, shows that there was 100% mycelia growth inhibition on *Rhizopus stolonifer* and *Aspergillus niger* respectively. This shows that Trichlorophenol has an active component that has a fungicidal activity against the growth of the tested fungi respectively. This means that Trichlorophenol has an active component that has a fungicidal action on the growth of *Rizopus stolonifer* and *Aspregillus niger* respectively. It could be deduced that the acid water and distilled water extracts have a fungistatic action against the fungicidal action of the Trichlorophenol in all the various concentration used; this apparently revealed that both the extracts and the standard drug have an active component that express Fungistatic and Fungicidal activity on the growth of the strains of fungi employed respectively. Moreover, a number of factors were identified to affect the activity of the extract such as the change in PH may not only affect the activity of the extract but also the rate of growth of the fungi cells and physiochemical state of their surface. A PH of 6.8 is optimal for the growth of many fungi cells and the rate of growth decline on either side of this range. The temperature of the extraction may also affect the activity of the extracts. At very high temperature some extract may lose their activity and may also produce different activity with various type of solvent, some are highly soluble in water while others are active in organic solvent and this may have affected the activity of the plant extract .Also since time of harvesting could not be ascertained and young flowering plant are better source of antimicrobial agent. (Sofowora, 2000) reported that the bulk of organic matter reduced the activity of plant antimicrobial agent hence advocated bulk of organic matter present in plant extract.

V. CONCLUSION

Based on the result of this study, *Zingiber officinale* produced antimicrobial substance that are probably responsible for it antifungal activity to be fungistatic against *Aspergillus niger* and *Rhizopus stolonifer* strain of fungi.

RECOMMENDATION

Hence the use of this plant root as a source of antifungal agents should be encourage and it could be said that there is need to seriously examine the active constituent in this plant along the line used by the herbalist and the mode of action of the plant. Antimicrobial agent need to be established; also the use of standard medicinal plant would therefore enable the government to reduce some of the much needed foreign exchange usually used for the importation of certain drugs. To achieve the much pronounced health for all in the dispensation, a bold attempt to use local refined medicinal plant in our hospital should be encouraged. Therefore the earlier we start using our indigenous herbs for drug production the better it will be for us in this millennium.

REFERENCES

[1]. Akinyanju, J.A., Owoyale, J.P. and Okanla, E.O. (2012).Anti microbial effect of leaf extracts of Acalypha torta in the State of medicinal plant research in Nigeria. J. Pharmacol**. 33** (1):247-251.

[2]. Boakye- yiadom, K. and Heman-Ackah, S.M. (2008).Cryptolepine hydrochloride effect on Staphylococcus aureus .J. Pharmacol. Sci.**68**:12.

[3]. Egawa, H., Tsutsuit , O.and Tatsu yama, K.(1976).Antifungal substance found in leave of Eucaliptus species.J.Expt.**33**:889-890

[4]. Ekundayo, O. (2006). In progress in essential oil research. Proceeding of the 16th international symposium on essential oil 2nd edition **3**(1):24-26.

[5]. Fage, O., El-said, F., Sofowora, E.A. and Hofer, J. (1989).An investigation into the efficacy of Ocimum gratissimum as used in Nigeria native medicine .J. Planta. Medical, **17**: 195-196.

[6]. Gbile, Z.O. and Adesina, S, K. (1986). Nigeria flora and its pharmaceutical potential. J.Med. **31**: 7-17.

[7]. Narian, A. and Satapathy, J.N. (2009).Antifungal characteristic of Vica rosea extract .J .Indan Phytophytology. **30**(1):36- 40.

[8]. Okanla, E.O., Owoyale, J.A. and Akinyaju, J.A. (1990).Trypanocidal effect of an aqueous extract of Acalyphahispida leave .Journal of Ethno Pharmacology.**29** (1):3

[9]. Oloke, P. and Kolawole, J.O. (2001). Development of Pharmaceutical from medicinal plant in Nigeria 2nd edition Pub.Med. Ltd .London: **120**-123.

[10]. Olorundare, O.E., Emudianugbe, T.S., Kuteyi, S.A. and Irobi, P.A. (1992).Antimicrobial activity of leave extract

ISSN No:-2456-2165

of [1 Cassia alata. J. Bio. Sci .Res .Communication **4**(2):113-117.
[11]. Oliver, B.(2008). Medicinal plant in Nigeria .2nd edition, J. Nig. Ant. & Sci.**3** (2):41.
[12]. Oyewole, M. O. (1991). Antimicrobial properties of some Nigeria medicinal plant .J. Pharmacol. **22** (4):1-17.
[13]. Oyewole, M .O. (2009). Antifungal effectiveness of the extract of Ocimum basilicum compared with standard antiseptic. J. Ethno. Pharmacol. **26**(3):1-2.
[14]. Sofowora, E, A. (1986).The state of medicinal plant Research in Nigeria .3rd edition, University of Ife printing press. Pp 13-15.
[15]. Sofowora, E.A. (1981).Man, plant and medicine in Africa some fundamental perspective. Inaugural lecture series 48.university of Ife press .Pp 132.