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Preliminary Screening of Some Nutraceuticals as Aflatoxin (*Aspergillus flavus*) Reduction Agents Using *In Vitro* growth Inhibition Technique

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Abstract:- The following nutraceuticals: *Moringa oleifera* Lam., Garlic (*Allium sativum*) and Ginger (*Zinger officinale*) were screened for their possible use as Aflatoxin (*Aspergillus flavus*) reduction agents by extracting their photochemical constituent's using aqueous and methanol extraction which were used as inhibitors of *A. flavus* . The results showed the presence of tannins, steroides, cardiac glycosides, anthraquinone, flavonoids, alkaloids terpenes and saponins in all the tested nutraceuticals. Methanol extraction had highest concentration of the phytochemical constituents in each and the growth inhibition zone of *A. Flavus* showed that the extracts of *Moringa oleifera* leaves, Garlic (*Allium sativum* L) and Ginger (*Zingiber officinale*) inhibit the growth of *A. flavus* in methanol extract, while aqueous extraction showed no zone of inhibition. The sensitivity results obtained revealed significant (p>0.05) difference on zones of inhibition at different concentrations of 12.5, 25, 50, 100 and 200mg/ml of the extracts of *Moringa oleifera* leaves (A), Garlic (B) and Ginger (C) and for methanol extracts (A), (B) and (C) at concentration of 12.5mg/ml. The highest zones of inhibition is extract (C) at 200mg/ml. The constituents in these nutraceuticals and the sensitivity results obtained indicates that these nutraceuticaals could be used as phytogenic feed additive for the inhibition of growth of mycotoxins in feeds

Keywords:- *Nutraceuticals, Aflatoxin, Aspergillus flavus, Invitro,*

I. INTRODUCTION

The contamination of foods and feeds with mycotoxins is a widespread phenomenon and thus providing ready access of mycotoxins reach to humans through food chain. The effect of ingested mycotoxins to human health varies from acute to chronic (Gbore, 2019). Similarly, Fung and Clark (2004) and Bunger *et al.* (2004) reported various health hazards caused by inhalation of dust containing mycotoxins. A variety of toxic effects associated with Aflatoxin contamination of the entire food supply chain is caused through post harvest activities and by inclusion of crops like maize and groundnut that are highly susceptible to infestation in monogastric diets.

Mycotoxin contamination of maize represents a widespread problem in the food and feed industries because maize can be easily contaminated by toxigenic mould such as *Aspergillus* and *Fusarium* species. These occur as either plant pathogens in the field or as the source of mycotoxin contaminants during storage as *Aspergillus flavusis is* one of the most abundant moulds that produce mycotoxins and contaminate human foods and animal feeds through fungal growth prior to and during harvest, or during improper storage (Binder, 2007; Ogara, *et al.,* 2017; and Gbore, 2019).

The impact of aflatoxin toxicity resulting from intake of mycotixin infested foods especially maize in Nigeria was noted when significant quantities of aflatoxins were detected in breast milk, implicated in neonatal jaundice and growth retardation (Atanda *et al.*, 2007; Bankole, *et al.*, 2006; Oyelami *et al.*, 1995) and the autopsy of brain tissues of children with kwashiorkor.

The use of some Nutraceuticals such as *Moringa oleifera* leaves, Garlic (*Allium sativum*) and Ginger (*Zingiber officinale*) to inhibit growth of microorganism and serve as important component of natural medical practices as the application of these herbs were reported to prevent morphological changes and oxidative damages to human and animals by enhancing the activities of antioxidant enzymes, reducing the density of lipid per-oxidation and inhibiting generation of free radicals (Fanelli, *et al.,* 1998; Ari *et al.,* 2012 and Ari *et al.* 2019).

The basic assumption in the current study was that utilization of these nutraceuticals will have an inhibitory effect on the growth of mycotoxins in maize, thus the objective of this study is therefore to evaluate the phytochemical constituent's of the nutraceuticals using different extraction medium and screen their effects on isolate of *Aspergillus flavus.* This process is a prelude to incorporation into a maize base broiler diets.

II. MATERIALS AND METHODS

- *Collection and Processing of Nutraceuticals*
- *Moringa oleifera Leave (MOL)*

Fresh *Moringa oleifera* leaves were harvested and air-dried under room temperature to prevent the leaves from being denatured until they are crispy to touch for 5 days, later the crispy was milled into fine powder (50 grams) and stored separately in an air tight polyethene bag as reported (Terzungwe, 2013;Hassan *et al.,* 2015; and Ari *et al.* 2019)

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- *Garlic (Allium sativum L) Bulb (GBM)*

Matured garlic bulbs were purchased from Vegetable Markets in Lafia, Nasarawa State. The scales around the fresh bulbs were removed while the bulbs were washed and rinsed properly in tap and sterile distilled water, respectively. The bulbs were sliced and ovum dried using hot ovum model DHG-9101- SA at the laboratory at 71ºC for 48 h. The ovum dried were grounded to smooth powder and stored in an air tight polyethene bag according to the methods described by Ari *et al*. (2012).

- *Ginger (Zingiber officinale) (GIM)*

Fresh ginger rhizomes were purchased from Lafia market, Nasarawa State. The cleaned rhizomes were chopped into tiny pieces and ovum dried in a hot ovum at 71ºC for 48 h. The materials are air dried and grounded to a smooth powder and stored in an air tight polyethene bag.

➢ *Phytochemical Screening of the selected Nutraceuticals*

The qualitative phytochemical analysis of the selected nutraceuticals were carried out using standard phytochemical techniques as described by Edeoga *et al*. (2005) and Oluduro (2012).

➢ *Isolate of Aspergillus flavus.*

The fungus of the isolate of *A. flavus* was collected from the Department of Dematopyhilosis, National Veterinary Research Institute NVRI Vom where the fungus was then sub-cultured using Sabouraud Dextrose Agar medium at the Microbiology Laboratory, of the same institution.

- *Preparation of spore suspension of the fungus*

Two weeks old purified cultures of *A. flavus* (when mycelia had produced spores) were used for preparing spore suspension. The spore suspension was prepared by adding sterilized distilled water (~1 ml) to each Petri-dish containing the fungus culture to allow the spores to be suspended in the water. The spore suspension was then collected and quantified using a spore counter; the concentration values obtained were adjusted to 1×10^6 spore per millilitre.

➢ *Assessment of anti-fungal activities of the Nutraceuticals*

Anti – fungal activities assessment was conducted using the paper disc diffusion method as described by Prabuseenivasan (2006). The assessments were done by measuring the growth inhibition zone of the fungus on Sabouraud Dextrose Agar medium. The extracts of MOL, GBM and GIM mg/ml respectively of the nuturaceuticals were prepared by dissolving the required amounts in dimethyl sulfoxide (DMSO). To remove any microbial contamination, the extracts were filtrated by using two-micrometer filters (Millipore filter 2) before submerging some autoclaved paper discs (6 mm in diameter) in the prepared concentrations in sterilized distilled water and/or in 0.2 percent fungicide Mancozeb solution. The discs were allowed to be saturated with the solutions while the submerged paper discs were then air dried. Petri dishes containing Sabouraud Dextrose Agar medium were inoculated with *A. flavus* by transferring half a millilitre of spore suspension of the fungus (1×10^6 spore/ml) and evenly spreading on the surface of the medium after which one paper disc loaded with each solution was placed in the centre of *A. flavus* inoculated Petri dishes.

The plates were incubated at 26°^{C} in the dark. The average diametrical growth inhibition zone of *A. flavus* around the paper discs impregnated with each samples were measured for 7 d post inoculation until the plates were completely covered with mycelia of the fungus.

➢ *Statistical analysis*

Data generated were subjected to one way analysis of variance and the means were separated using Duncans Multiple Range Test using SPSS software version 22

III. RESULTS AND DISCUSSION

The phytochemical evaluation as indicated in Table1 showed that the active constituents found in *Moringa oleifera* leaves, Garlic and Ginger the presence of tannins, steroides, cardiac glycosides, anthraquinone, flavonoids, alkaloids terpenes and saponins. These constituents differ in concentration between extractions medium. The extract for solvents methanol extract showed more number of constituents when compared with aqueous extract. This agrees with the report of Divya *et al*. (2016) who worked on the phytochemical composition of Garlic (*Allium sativum*), and the report of Chinwe *et al.* (2015) who reported more constituents in *Moringa oleifera* leaves in aqueous solvent than in methanol extract. Similarly, more phytochemical constituents were extracted using methanol. These bioactive constituents in *Moringa oleifera* leaves, Garlic and Ginger have been used in the treatment of many diseases and disorders (Shipra *et al.,* 2012; Chinwe *et al.,* 2015; Divya *et al.,* 2016) as well as in the development of resistance against microbial pathogens

The results in (table 2) shows zones of inhibition at different concentrations of 12.5, 25, 50, 100 and 200mg/ml of the extracts of *Moringa oleifera* leaves (A),Garlic (B) and Ginger (C). the results showed a significant ($P<0.05$) difference among the three nutraceticals and the concentration levels whilst methanol extracts (A), (B) and (C) at concentration of 12.5mg/ml showed for extract (A) zones of inhibition (mm)1.70 ± 0.01 followed by extract (C) with 1.60 ± 0.01 while the least zone of inhibition is extract (B) with 1.10 ± 0.01 at 25mg/ml . Methanol extracts (A) 1.80 ± 0.01has the highest zones of inhibition while the least has1.35 ± 0.01.

At 50mg/ml (A) and (B) has the highest 1.80 ± 0.01 zones of inhibition (mm) each while the least is (C) with 1.60 ± 0.01, However with increase at 100mg/ml methanol extract (C) has the highest zone of inhibition of 1.95 ± 0.01 followed by methanol extract of (A) while the least zone was extract of (B). The highest zones of inhibition is extract (C) with 2.65 ± 0.01 at 200mg/ml followed by extract (A) 2.60 ± 0.01 while the least zones of inhibition was extract (B)1.95 ± 0.01.

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The results also revealed that all the aqueous extracts A, B and C of the nutraceuticals showed no inhibition zones. These indicate that aqueous extracts did not have any positive inhibitory effect and therefore only methanol extracts of the nutraceuticals have significant impact on the mycotoxins. Similar constituents extracted from these nutraceuticals were reported to be used for the treatment and prevention of various diseases and disorders in both human and animal food and feeds (Mikail, 2010; Tiwari *et al.*, 2011; Shipra *et al.*, 2012).

The growth inhibition zone of *A. flavus* shown in (fig.1) indicated that the extracts of *Moringa oleifera*leaves, Garlic (*Allium sativum* L) and Ginger (*Zingiber officinale*) inhibit the growth of *A. flavus* in methanol extract, but no zone of inhibition in aqueous extract was noticed. This is in agreement with the reports of Jafar and Sahar (2015) who used essential oil of *Thymus eriocalyx* on the growth of *A. flavus* and Jun *et al.*(2012) who use the essential oil extracted from the bark of *Cinnamomum jensenianum* for the same objective.

IV. CONCLUSION

The inhibitory effects of the methanol extracts of the nutraceuticals studied indicates the possibility of their use as alternative prebiotic control of *A. flavus* which are often associated with deterioration and contamination due to storage conditions and other activities on grains and commercial poultry feeds.

The results of this investigation show that methanol extraction of ginger (*Zingiber officinale*) at 200mg/ml concentration has the best inhibitory effect on the growth of *A. flavus* among the three nutraceuticals.

Parameter	*Moringa oleifera* leaves		Garlic		Ginger	
	Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol
Tannins	+	+	+	-	-	+
Steroids	+	+	+	+	+	-
Cardiac glycoside	+	+	+	+	+	+
Anthraquinone	+	-	-	+	-	+
Flavonoids	+	+	+	+	+	+
Alkaloids	+	-	-	+	-	+
Terpenes	+	+	+	+	+	+
Saponins	+	+	+	+	-	+

+ indicated presence, - indicated absent

Table 1:- Phytochemical Constituents of Some Nutraceuticals

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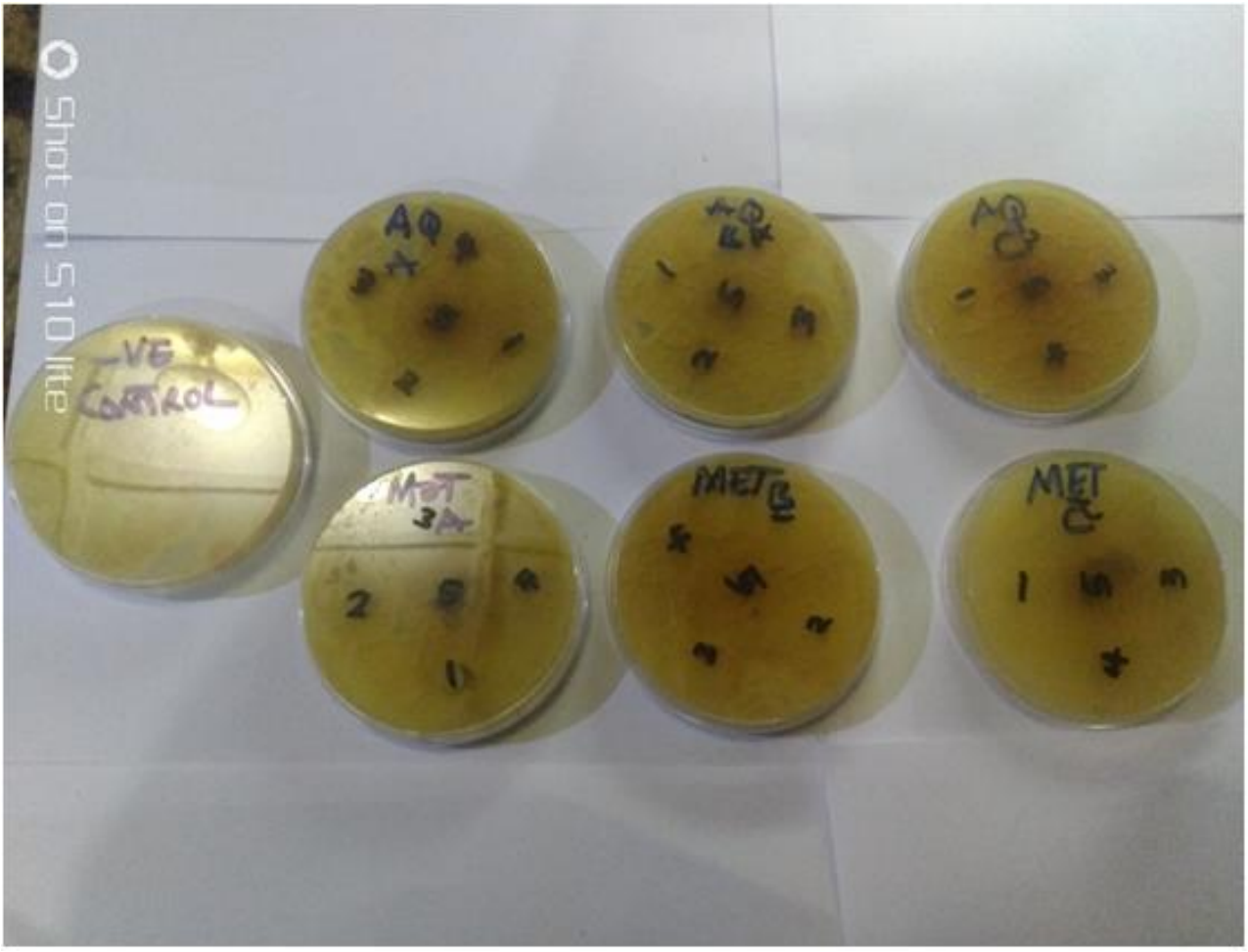

Fig 1:- The Invitro growth inhibition zone of *Aspergillus flavus* onSabouraud Dextrose Agar medium resulting from treatment of the fungus with 12.5, 25, 50, 100 and 200mg/ml extracts of methanol and aqueous of *Moringa oleifera* leaves (A),Garlic (B) andGinger (C).

	Concentration/Zones of inhibition (mm)				
Sample extract	**12.5**	**25**	**50**	**100**	**200**
Meth. Extract of (A)	1.70 ± 0.01^{a}	1.80 ± 0.01^{a}	1.80 ± 0.01^{a}	1.55 ± 0.01^{b}	2.60 ± 0.01^{b}
Meth. Extract of (B)	1.10 ± 0.01^{c}	1.60 ± 0.01^{b}	1.80 ± 0.01^{a}	1.20 ± 0.01^{c}	1.95 ± 0.01^{c}
Meth. Extract of (C)	1.60 ± 0.01^{b}	1.35 ± 0.01^{c}	1.60 ± 0.01^{a}	1.95 ± 0.01^{a}	2.65 ± 0.01^{b}
Aq. Extract of (A)	-	-	-	-	-
Aq. Extract of (B)	-	-	-	-	-
Aq. Extract of (C)	-	-	-	-	-
Significant	**	**	**	**	**

Table 2:- Sensitivity results showing zones of inhibition at different concentration

^{abc} Means on the same column bearing different superscripts are significantly different at ($P<0.05$), Means inhibition (mm), ± standard error of mean, - no inhibition, **significant at 95%, Meth. (Methanol), Aq. (Aqueous),*Moringa oleifera* leaves (A),Garlic (B) andGinger (C)

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