



EFFECTS OF *ALOE VERA* (L.) BURN F. EXTRACT ON *ASPERGILLUS FLAVUS* AND *ASPERGILLUS NIGER* ISOLATED FROM GROUNDNUT (*Arachis hypogea* L.)

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Abstract

Arachis hypogea is a species of flowering plant in the family Fabaceae. It is infected by *Aspergillus flavus* and *Aspergillus niger* in all stages of growth, storage and in transit. These fungi cause spoilage, loss by contaminating seeds; cause aflatoxin contamination which are carcinogenic and hepatotoxic. Groundnut infected by these fungi may cause aspergillosis in immune-compromised individuals. In this study the antifungal efficacy of unfractionated whole *Aloe vera* extract on *A. flavus* and *A. niger* isolated from healthy and deteriorated groundnuts (obtained from New Benin and Oba Markets (Ring Road) was investigated. Bioassay of the *Aloe vera* was done by the pour plate method. The results showed total inhibition of the *A. flavus* isolated from healthy groundnut at 100, 75, 50 and 25% concentration respectively, for New Benin market; a high inhibition of the *A. flavus* isolated from the deteriorated New Benin groundnut at the 100 and 75 % concentration respectively and a moderate inhibition at 50 and 25%. There was no significant inhibition at all concentrations of the *A. niger* isolated from the healthy groundnuts for Oba market. Phytochemical analysis of the *A. vera* used showed the presence of Alkaloids, phenols and Tannins which validates the antifungal properties of *A. vera*. The negative result obtained in the bioassay against *A. niger* may be that the concentration of *A. vera* was not sufficient to cause an effective inhibitory action against its growth. Further work is ongoing to determine the extract concentration that would effectively inhibit *A. niger*.

Keywords: *Aloe vera*, Phytochemicals, *Aspergillus flavus*, *Aspergillus niger*, *Arachis hypogea*

Introduction

Naturally occurring biochemical compounds (Phytochemicals) of plants which are obtained by chemical or mechanical extraction are known as Plant extracts (Okungbowa *et al.*, 2012). These extract contribute a great deal in opposing the pathogenicity of microorganism (Vyvyan, 2002).

Aloe vera is a member of the liliaceae family. *Aloe* species numbers over 300 of which *A. vera* L. is the most widely recognized and utilized for various medical and cosmetic purposes (Surjushe *et al.*, 2008; Joseph *et al.*, 2010).

A bitter yellow liquid exudates of high phenolic content and a mucillagenous pulp are the main parts obtainable from the leaves of *A. vera*. The liquid fraction constituents are largely phenolic in nature (Reynolds, 1985). Carbohydrate polymers such as glucomannans and pectic acid including some other organic and inorganic content can also be obtained from the pulp. Newall *et al.* (1986) reported that *A. vera* contains some phytochemicals such as saponins, tannins and other substance such as mono and polysaccharides, sterols, organic acids, enzymes, vitamins and minerals.

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Groundnut native to South America, Mexico and Central America is an herb grown annually (Rashmi *et al.*, 2017). In Nigeria, other than palm oil, it is one of the most important and generally accepted source of cooking oil compared to other plant sources. Most farmers and agro-merchants store groundnuts in time of excess to resale at the time of shortage. However, these stored legumes get infected by some species of the genus *Aspergillus* which produces a wide range of metabolite, one of such is Aflatoxins.

A good deal of fungi which may be identified by fungal spores: that give characteristic coloration, inhabits soil and many of them infect and damage the seed and germinating seedlings of groundnut. Nuts are susceptible to contamination by *A. flavus* and *A. niger* during the pre and post storage phase and on transit. In groundnut, fungi infect the leaves, seedlings and cause rots of seeds thereby causing diseases. Some of the diseases of the seedlings caused by these fungi are: root rot, stem rot, wilts, blight and pod rot; that of the leaves are: rust, early and late leaf spots

Aspergillus niger has been implicated to be pathogenic in studies carried out by Sharma, (2012). It causes the Crown rot of peanuts which is characterized by root curling and deformation of the upper parts of the plant. *Aspergillus niger* is also reported to produce ochratoxin A, fumonisin B2 and aflatoxins (Abraca *et al.*, 1994; Schuster *et al.*, 2002; Noonimabc *et al.*, 2009; Al-Abdalall, 2009) in stored commodities. The fungus, *Aspergillus flavus*, produces aflatoxin and causes aflaroot or yellow mold of groundnut. Affected seeds shrivel up, dry and are covered by spores that are yellow or greenish in colour. The cotyledons become necrotic with margins that are reddish brown; seedlings are highly retarded in growth, the leaves are pale and light green in colour with a reduced size (Pal *et al.*, 2014).

Antibiotics, antibiosis and chemicals have been used to treat *Aspergillus niger* infections. However, Biological control has been found to be the best and most effective treatment (Sharma, 2012). In this study, the antifungal potentials of unfractionated extract of *Aloe vera* on *A. niger* and *A. flavus* isolated from fresh and deteriorated groundnut was investigated and the extract concentrations that can inhibit the mycelia growth and development of this species was determined.

Materials and Method

Preparation of media

39 grams powder of Potato Dextrose Agar (PDA) in one liter of deionizer water was soaked for 10mins according to standard laboratory protocol. The mixture was swirled to mix and sterilized at 121°C for 15 minutes; it

was then allowed to cool to 47°C and mixed well before pouring into Petri dishes.

Isolation of micro organism

Tissue maceration method was used to isolate organisms and stock solution prepared. Cultures were obtained by using the serial dilution and pour plate method; Chloramphenicol at 0.02 mg per 200ml of medium was introduced at pouring to inhibit the growth of bacteria. Sub-culturing of the isolates was done; pure isolates obtained was streaked into nutrient agar slants in a McCartney bottle and stored in the refrigerator at 4°C. The isolated fungi were identified using the criteria adopted by Raphel and Fennel 1965; Samson *et al.*, 2002.

Preparation of Plant extract

The *A. vera* was oven dried at 50°C. A soltux extractor was used to extract the gel which was then concentrated with a rotary evaporator

Phytochemical analysis of Plant extract

The detection of the phytochemical present in the *Aloe vera* extract was done using the official methods of analysis. (A.O.A.C, 2005)

Bioassay of Plant extract

Spore suspension of the pure cultures of the isolates was prepared by using a flamed wire loop to scoop a portion of the pure isolates into 4mls of water that was sterilized at 121°C for 15 minutes to dislodge the spores into the sterile water. 0.5mls each of the spore suspension was then pipetted with a 5mls syringe into Petri dishes which were labeled New Benin 100%, New Benin 75%, New Benin 50%, New Benin 25% and New Benin control. This was also repeated for all the isolates obtained from Oba market respectively in duplicate. 1ml of the plant extract was then pipetted into each of the spore suspension in the Petri dishes, this was then followed by PDA using the pour plate method. The Petri dishes were then kept at room temperature for 5 days.

RESULTS

Table 1: Percentage composition of the phytochemicals present in *Aloe vera* used for this research work

Phytochemicals	Percentage composition (%)
Tannins	0.54
Saponins	0.011
Flavonoids	0.44
Alkaloids	0.66
Phenols	0.32

Table 2: *Aspergillus* species isolated from *Arachis hypogea* isolated from groundnut obtained from two locations.

Locations	Morphological description	Suspected organisms
New Benin Market (deteriorated)	Black mycelia growth	<i>Aspergillus</i>
	Light green mycelia growth	<i>Aspergillus</i>
New Benin Market (fresh)	Black mycelia growth	<i>Aspergillus</i>
	Light green mycelia growth	<i>Aspergillus</i>
Oba Market, Ring road (deteriorated)	Black mycelia growth	<i>Aspergillus</i>
	Light green mycelia growth	<i>Aspergillus</i>

The isolated fungi were identified using the criteria adopted by Raphael and Fennel 1965; Samson *et al.* (2002).

Discussion

Groundnut is an important food crop and oil seed in Nigeria. It is one of the crops that contributes to the gross domestic product (GDP) of the country: Nigeria is the fourth largest producer in the world and the highest producer in Africa with 1.55 million metric tons (FAOSTAT, 2014). World trade in groundnut has declined since the 1990's, reason being that there has been a decline in the export from developing countries. (FAO, 2002; Revoredo and Fletcher 2002; Sowley, 2016)

Aflatoxin contamination of groundnut and its by-product is a culprit for these decline: standard for aflatoxin content has been set by the main importers and these standards are often not met by developing countries (Sowley, 2016). Research is being carried out to discover the best method of forestalling contamination from aflatoxin in food crops; the use of biocontrol with the utilization of phytochemicals from plant extract is one major area which is critically being looked into.

The antimicrobial potentials of most plants can be attributed to the phytochemicals imbedded in them. These phytochemicals are secondary metabolite used by these plants to protect themselves from predators; they also have diverse use in medicine and pharmaceuticals. The presence of phenol, lignin and saponins confers

antifungal properties on plants whose extract has been used as biocontrols.

Several authors have investigated the efficacy of plant extract and essential oils as antifungal agent. Iraj *et al.* (2006) investigated the inhibition of growth and the morphological alterations of *A. niger* by essential oils from *Thymus eriocalyx* and *Thymus x-porlock* and reported that the essential oils from these plants could be safely utilized as a preservative. Sitaria *et al.* (2011) investigated the antifungal efficacy of *A. vera* gel against plant pathogenic fungi and concluded that the gel of *Aloe* completely inhibited the growth of *Drechslera hawaiiensis* and *Alternaria alternata*.

In this present investigation, the in-vitro antifungal efficacy of *A. vera* on *Aspergillus flavus* and *Aspergillus niger* was investigated and results showed that the *Aloe vera* extract significantly inhibited the growth of *A. flavus* at 100% and 75% with moderate inhibition was recorded at 50% and 25% concentration compared to the control. There was a significant inhibition of all the *A. flavus* isolate compared to the *A. niger* in which there was no significant inhibition at all concentration. The insignificant inhibition of the extract against *A. niger* correlates with the result of Sitara *et al.* (2011) who reported that *Aloe vera* possess remarkable antifungal activity towards all fungi compared to the control except *A. niger* when the antifungal activity of *Aloe vera* gel against plant pathogenic fungi viz *A. niger*, *A. flavus*, *Alternaria alternata*, *Drechlera hawaiiensis* and *Penicillium digitatum*. The extract showed complete inhibition at all concentration on the *A. flavus* isolated from fresh groundnut than the isolates from deteriorated groundnut. The presence of the fungi in the fresh groundnuts may be that before harvest and /or during transits, some of these groundnuts could have been infected; the high efficacy of the extract on the isolates of the fresh than the deteriorated groundnut may be that the infection of the pathogenic fungi is more severe in the deteriorated state than in the fresh groundnut. The extract can be tested as a coating to prolong the time of spoilage of the groundnut just as been done by Misir *et al.* (2014) where *Aloe vera* gel was used as a novel edible coating for fresh fruit. *Aloe vera* can also be used as a preservative, just as been done by Serrano *et al.* (2006) where grapes at 1°C coated with the gel of *Aloe* could be preserved for 35 days against 7 days of untreated grapes. All the plates in which there was a positive result on the effect of the extract was kept at ambient temperature for an extra 7 days in addition to the 7 days from which the result were taken, it was

observed that the plates looked the same as that of the result taken the previous week.

CONCLUSION

The result obtained from this present study confirms the antifungal efficacy of *Aloe vera* extract against *Aspergillus flavus* isolated from *Arachis hypogea*, while there was no inhibition of the *A. niger* isolates at all concentration: the inhibition of the *A. flavus* isolates at both locations may be that the concentration of the *A. vera* extract was sufficient to inhibit its growth and not sufficient to cause an effective inhibitory action against the growth of *A. niger*.

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