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Full Length Research Paper

Antifungal efficacy of crude extracts of *Azadirachta indica* and *Vernonia amygdalina* against pathogenic Aspergillus niger (ATCC 16404)

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This research work was conducted to assess the potency of ethanolic and hot water extract of *Azadirachta indica* and *Vernonia amygdalina* against pathogenic *Aspergillus niger* (ATCC 16404). Potato Dextrose Agar was the medium under which the antimicrobial sensitivity test was carried out. Streptomycin solution was also added to the medium to selectively inhibit the growth of bacterial cell. The results showed that, higher concentration of the extracts of the two plants species were efficient in stifling the growth of *A. niger*. This was evident as the inhibition zones increased with increasing concentration of the extracts. The results also showed that ethanolic extract of *A. indica* and *V. amygdalina* is a bit potent than the hot water extracts against the fungus species ATCC 16404. The results revealed that, the areas of inhibitions at the same concentrations were found to be slightly high in ethanolic extracts than that of hot water extracts.

Key words: Antifungal, Azadirachta indica, Vernonia amygdalina, Aspergillus niger.

INTRODUCTION

Contamination of stored food by fungi species especially *Aspergillus* species has become a global problem. This problem is more disturbing in the tropics especially in West Africa where the climatic conditions favor the proliferation of these opportunistic fungi. *Aspergillus niger* is highly cosmopolitan due to its wide range of temperature and pH tolerance (Perrone et al., 2007; Perfect et al., 2001).

A. niger is notorious for its saprophytic behavior in the soil. It is responsible for black mould disease in garlic, onion and shallot; root stalk rot of Sansevieria; and boll rot of cotton. This fungus causes spoilage of cashew dates, kernels, figs, and dried prune. Groundnut crown rot disease is also caused by *A. niger* (Bobbarala et al., 2009; Panchal and Dhale, 2011). These diseases that infest stored food end up in the gut of human and animals where it manifests several illnesses.

Controlling fungi species that cause havoc in stored food have its own controversies. Chemical pesticides usage is a popular way to control various plant diseases as compared to the natural means which deals with extracts from plants or plant parts.

Consumers are now much concern about the level of synthetic fungicides, because of their non-biodegradability

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Author(s) agree that this article remain permanently open access under the terms of the <u>Creative Commons Attribution</u> <u>License 4.0 International License</u> nature. Many also argue that they are unfriendly to nature as they may affect untargeted beneficial organisms such as *Rhizobium* species which are natural nitrogen fixers. Several studies have revealed that plant extracts can be an alternative means of controlling fungi as natural pesticides (Arokiyaraj et al., 2008; Gangadevi et al., 2008; Brindha et al., 2009).

Ogo-Oluwa and Kator (2016) conclude from their research findings that *Vernonia amygdalina* possess inhibitory effect on rot causing fungi of tomato in storage and suggest it could also extend shelf life of the product. Research findings credited to Subbarao and Anna (2015) reveal that, neem supplements treated with manure is an inexpensive way to prevent pre-harvest contamination of EcO157 as they established that supplementing neem leaf and bark to manure resulted in elimination of pathogenic EcO157 in less than 10 days.

V. amygdalina, a member of the Asteraceae family, is a small shrub that grows in tropical Africa to a height of 2 to 5 m (6.6-16.4 ft). The leaves are elliptical and up to 20 cm (7.9 inch) long (Igile et al., 1995). The leaves are dark fleshy and green colored with a characteristic odor and a bitter taste. The species is indigenous to tropical Africa and is found wild or cultivated all over sub-Saharan Africa. The leaves are eaten, after crushing and washing thoroughly to remove the bitterness. All parts of the plant are pharmacologically useful. The cooked leaves are a staple vegetable in stews and soups of various cultures throughout equatorial Africa (Mayhew and Penny, 1998). V. amygdalina (Bitter leaf) is consumed either as a vegetable for cooking African dishes or the aqueous extract could be drank as tonics for the treatment of various ailments (Kigigha and Ebubechukwu, 2015; Imaga and Banigbetan, 2013). The bitterness is realized to be due to factors such as the presence of alkaloids, saponins, tannins and glycosides which have been shown by various authors to be present in bitter leaf (Butler and Bailey, 1973; Bonsi et al., 1995). Kambizi and Afolayan (2001) reported that acetone extract of V. amygdalina possesses antibacterial activity towards Bacillus cereus, Bacillus pumilus, Bacillus subtilis, Micrococcus kristinae. Staphylococcus aureus, Enterobacter cloacae and Escherichia coli.

Azadirachta indica (neem) is used in traditional medicine as a source of many therapeutic agents in the Indian culture and dwells well in the tropical countries. Its twigs provide a chewing stick and are wildly used in the Indian sub-continent (Almas and Ansal-Lafi, 1995). More than 135 compounds have been isolated from different parts of the tree. They have been divided into isoprenoid and non-isoprenoid compounds (Kumar and Parmar, 1996). Neem has been used to control insect pest and diseases, as chewing stick and the water believed to be used as tongue cleaner (Birgit, 2013). Products of neem trees have been used in India for over two millennia for their medicinal properties, neem leaves have also been used to treat skin diseases like eczema and psoriasis

(Anna, 2006). Moreover, the oil's antifungal, antibacterial, moisturizing and soothing potential permit its usage (Birgit, 2013).

This research work focused on assessing the best extract concentration of *A. indica* and *V. amygdalina* that is potent enough to curb the development of *A. niger* as a mycotoxin producing species.

MATERIALS AND METHODS

Collection of plant and test organism

A. indica and *V. amygdalina* were collected from the farms within Navrongo, Kassena Nankana Municipality in Upper East Region of Ghana. The pathogenic strain ATCC 16404 of *A. niger* was obtained from Food Research Institute Laboratory, Accra.

Extract preparation

Two separate extracts were prepared as the following.

Preparation of hot aqueous extract

Fresh leaves of plant samples were thoroughly washed under running tap. The leaves were then air-dried for two days. The dried leaves were blended into powder. Powdered samples (5, 4, 3, 2, and 1 g) were weighed separately into five volumetric flasks each containing 10 ml of sterile water. Concentration of 500, 400, 300, 200 and 100 mg/ml were made. The test tubes were then placed in water bath and heated for 1 h at 80°C in order not over heat the extract. The extracts were left overnight after which they were filtered into sterile test tubes using Whatman No. 1 filter paper (110 mm). The residue obtained was kept inside aluminum foil and the extracts were stored in refrigerator at a very low temperature (4°C) before use.

Preparation of ethanolic extract

Powdered samples (5, 4, 3, 2, and 1 g) were weighed separately into five test tubes each containing 10 ml of 75% ethanol which yielded respective concentrations of 500, 400, 300, 200, and 100 mg/ml. This was done to obtain various concentrations which will inhibit the fungi. The extracts were left over night after which they were filtered into sterile test tubes using Whatman No. 1 filter paper (110 mm). The filtrate obtained was evaporated to dryness at 45°C and the residue obtained was kept inside aluminium foil. The extracts were stored in refrigerator at a very low temperature (4°C) before use

Preparation of griseofulvin (Control)

Powdered Griseofulvin (5, 4, 3, 2, and 1 g) were weighed separately into five sterile test tubes each containing 10 ml of sterile distilled water which yielded concentrations of 500, 400, 300, 200 and 100 mg/ml.

Media preparation

Potato Dextrose salt agar (39 g) was dissolved in 1 ml of distilled

Concentration levels	Zones of inhibition (mm)			
(mg/ml)	Hot aqueous extract of leaf	Ethanol extract of leaf	Control (Griseofulvin)	
500	18.06±0.20	20.30±0.53	24.73±1.10	
400	14.23±0.31	17.23±5.72	20.20±0.53	
300	11.10±0.68	15.40±0.50	16.97±0.42	
200	8.07±0.50	10.06±0.43	13.00±0.61	
100	-	8.16±0.31	11.00±0.36	

Table 1. Antifungal activity of dry Azadirachta indica leaves on Aspergillus niger.

 Table 2. Antifungal activity of dry Vernonia amygdalina leaves on Aspergillus niger.

Concentration levels	Zones of inhibition (mm)		
(mg/ml)	Hot aqueous extract of leaf	Ethanol extract of leaf	Control (Griseofulvin)
500	19.00±0.10	21.87±0.35	24.73±1.10
400	16.10±1.63	18.03±0.10	20.20±0.531
300	11.37±0.47	13.73±0.38	16.97±0.42
200	9.16±0.38	10.43±0.78	13.00±0.6
100	-	-	11.00±0.36

water. The mixture was stirred using magnetic stirrer and autoclaved at 121°C for 15 min. The media was cooled to 45°C to eliminate bubbles. 1 ml of 100 mg of streptomycin solution was prepared and added to the media to selectively inhibit the growth of bacterial cells. The prepared media was poured into the Petri dishes under sterile condition of lamella flow chamber to avoid contamination. Bubbles were eliminated from the poured media by flaming using Bunsen burner.

Inoculation of test organism (A. niger)

The test organism (*A. niger*) was inoculated using sterile inoculating loop under aseptic conditions. The loop was used to touch the freshly grown spores and transferred onto Petri plate containing sterile PDA. The test organism was streaked on the plate to ensure uniform spreading on the Petri plates.

Antimicrobial sensitivity test (Agar Well Diffusion Test)

A cork borer (6 mm in diameter) was sterilized by flaming and used to create four wells on each of the inoculated plates. The holes created were then filled with the plant extracts of different concentrations (100, 200, 300, 400 and 500 mg/ml). The plates were allowed to stand for 1 h for pre-diffusion of the extract (Esimone et al., 1998) and incubated at 25°C for 3 days. At the end of the incubation period, the plates were examined for the measurement of zone of inhibition (Hugo and Russel, 1996). The same sizes of holes were bored in which the control solutions of different concentration were incorporated. After a period of 3 days, the plates were examined and zones of inhibitions were measured by taking the distance within the zone across the well.

RESULTS

Antifungal activity of dry A. indica leaves on A. niger

The test organism was susceptible to the antifungal-

control (Griseofulvin) at all the concentration levels. The first concentration showed a wider zone of inhibition (24.73 mm), followed by 20.20, 16.97, 13.00 and 11.00 mm at concentrations 500, 400, 300, 200 and 100 mg/ml, respectively. *A. niger* was also susceptible to ethanol extract at all the concentration levels, that is, 500, 400, 300, 200 and 100 mg/ml which gave inhibition zones of 20.30,17.23,15.40,10.06 and 8.16 mm, respectively. The aqueous extract exhibited the least zones of inhibition. *A. niger* were resistant at 100 mg/ml concentration (no inhibition zone was measured). Concentrations 500, 400, 300, and 200 mg/ml revealed inhibition zones of 18.06, 14.23, 11.10 and 8.07 mm, respectively shown in Table 1.

Antifungal activity of dry *V. amygdalina* leaves on *A. niger*

Table 2 shows the antifungal activity of *V. amgdalina* (Bitter leaf) extracts against *A. niger*. The test organism was susceptible to the antifungal (Griseofulvin) at all the concentration levels. The first concentration showed a wider zone of inhibition, that is, 24.73 mm, followed by 20.20, 16.97, 13.00 and 11.00 mm at concentrations of 500, 400, 300, 200 and 100 mg/ml, respectively. *A. niger* was also susceptible to ethanol extract at all the concentration levels, that is, 500, 400, 300, and 200 mg/ml, excluding 100 mg/ml which gave inhibition zones of 21.87, 18.03, 13.73, and 10.43 mm and no inhibition zone, respectively. The aqueous extract exhibited the least zones of inhibition and moreover, *A. niger* was resistant at 100 mg/ml concentration without an inhibition zone. Concentrations of 500, 400, 300, and 200 mg/ml

have shown inhibition zones of 19.0, 16.10, 11.37 and 9.16 mm, respectively.

DISCUSSION

The zones of inhibition showed that ethanolic extraction of both plants resources is superior to hot aqueous extraction in keeping *A. niger* inactive to proliferate. This finding is collaborated by research conclusions drawn by Susmith et al. (2013), Imaga et al. (2013) and Akah and Okafor (1992) who found neem and bitter leaves extract to contain alkaloids, tannins, flavonoids, steroids and saponins among others. These are also the active ingredients in plants that exert antimicrobial activity through different mechanisms (Igbinosa et al., 2009).

phytoconstituents These alkaloids. flavanoids. glycosides, and saponins act as antifungal principles of plants. One of the most common biological properties of alkaloids is their toxicity against cells of foreign organisms. These phytochemical compounds exert antimicrobial activity through various mechanisms and the secondary metabolites in them are known to be biologically active and therefore play significant roles in bioactivity of medicinal plants because the medicinal values of medicinal plant lies in these phytochemical compounds which produced a definite and specific action on the human body (Kam and Liew, 2002). These phytochemical compounds are actually the defensive mechanism of the plants against different pathogens (Hafiza, 2000; Faiza aslam et al., 2009).

High concentration of the plant extracts could confer greater inhibitory effect from both extraction methods as evident in the results. This could be due the presence of much of several alkaloids efficiently resisting the growth of the fungi. Lower concentration of the extracts will also result in lesser inhibitory effect. This was also observed in the report by Esimone et al. (1998) which says that extract of plants inhibit the growth of various microorganisms at different concentrations. This was same as the results recorded in the present study where the increase in the concentration of extracts corresponds with the increase of the diameter of inhibition zones.

Conclusion

From the study, it could be concluded that both hot aqueous and ethanol extraction of the plants conferred inhibitory effect, but ethanolic extract yielded a greater effect of inhibition. The control had a greater zone of inhibition than both plants extracts.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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