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ANTIFUNGAL ACTIVITIES OF COLUMN CHROMATOGRAPHIC FRACTIONS OF METHANOLIC AND N-HEXANE LEAVES EXTRACT OF *Senna occidentalis* AND *Boswellia dalzielii* ON SOME CLINICAL FUNGAL ISOLATES

Hauwa Bello Abubakar and Aisha Umar

Faculty of Science, Sokoto State University, Sokoto

ABSTRACT

Plants notably *Senna occidentalis,* and *Boswellia dalzielii*, have been documented to be effective against fungal species, this work is aimed at separating various fractions of the selected plants leaves extract using chromatographic techniques and to also assess the column chromatographic fraction of each of these plants for antifungal activities. The chromatographic fractions of methanol and n- hexane leaves extracts of *Senna occidentalis* and *Boswellia dalzielii* was determined. The column fractions revealed five fractions in each extracts of both plants (n- hexane fractions 1to 5 and methanol fractions 1 to 5). The antifungal activity of column chromatographic fractions of n- hexane leaves extract of *Senna occidentalis* indicated that, both the fractions were active against *C. albican, and C. pseudotropicalis* at concentration of 10, 20 and 30 mg/ml, and showed little

Introduction:

Plants are healthy and natural resource of life (Vivek et al., 2016). In particular, medicinal plants are of great importance with endless therapeutic properties useful for curing various diseases with an advantage of being natural (Vivek et al., 2016). At present, there are un-countable products in market, with adverse side effects on once heath. Therefore, the use of secondary metabolites from plant origin could be an advantage and best solution to narrow down the use of unhealthy products (Vivek et al., 2016). In past, the plant or microbial extracts in crude

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effect against *A. flavus* with no activity against *A. niger*. Analysis of variance was conducted and the results indicated that there was no significant difference between the different fractions of the plants tested against organisms. The result of the antifungal activity of column chromatographic fractions of methanolic extracts of *Senna occidentalis* indicated high antifungal activity against *C. albican, C. pseudotropicalis* at both concentrations of 10, 20, and 30mg/ml, with little effect against *A. niger* and *A. flavus* especially at 10mg/ml concentration. The result of the antifungal activity of column fractions of n- hexane extract of *Boswellia dalzielii* indicated that n- hexane fractions 1-5 showed highest activity against *C. albican* and *C. pseudotropicalis* but with little activity against *A. flavus* and no activity against *A. niger*. Analysis of varience was conducted which reveal that there is no significant difference between the tested plants against organisms.The antifungal activity of column chromatographic fraction of methanol leaves extract of *Boswellia dalzielii* revealed high activity against *C. albican* and *C. pseudotropicalis* at various concentrations used. High activity was also recorded against *A. niger* at concentration of 20 and 30 mg/ml, with less activity recorded against *A. Flavus*. Analysis of varience was carried out and the results revealed that there is no significant difference between the plants tested against organisms. From the results obtained in this work, it is apparently cleared that both the fractions of the two plants were active against the tested isolates especially the methanolic extract of both plants.

Keywords: *Medicinal Plants, ANTIFUNGAL, Senna Occidentalis, Boswellia Dalzielii*

Or partially-purified forms were the only sources of medication available for the treatment of human and animal diseases. This gave an idea that the effect of a drug in human body is due to an interaction of drug with biological molecules. This opened new doors in pharmacology, as pure, isolated chemicals, instead of extracts, as the standard for the treatment of diseases. At present, there are innumerable number of such bioactive compounds isolated form crude extracts and

their chemical structure was elucidated (Vivek et al., 2016). Moreover, plants have always been a source of a wide array of secondary metabolites with potential pharmacological properties (Vivek et al., 2016). Polyphenolic (flavonoids) compounds occur ubiquitously in foods of plant origin have many beneficial health effects.

Traditional medicine is the oldest method of curing diseases and infections and various plants are use in different parts of the world to treat human diseases and infections (Venugopal and Venugopal, 1994).Plants are among the most important and common sources of potentially valuable new drugs, it has been used for centuries to treat infection and other illness in native community but controlled clinical studies are scarce. The use of medicinal plants is very wide spread in many parts of the world, Nigeria inclusive (Kunle, 2000).

In Nigeria, many plants are used against infectious diseases, (Agassounon 2011). Plant base drugs are gaining popularity because of several advantages such as fewer side effect, better patient tolerance, relatively less expenses and acceptance due to a long history of use, especially herbal medicine has provide rational means for the treatment of many diseases that are incurable in other system of medicine. (Abdullah and Lawal, 2010). Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical diseases (Fabricant *et al.,* 2001). Many plants especially those used by traditional healers produce pharmaceutically active compound that have antimicrobial, anthelminthic, antifungal, antiviral, anti-inflammatory, anti-oxidant and anti-diabetic activity (Rabah *et al.*, 2007, Gupta *et al*, 2007 and Karim *et al.*, 2011).Plant possess a naturally occurring component that give it color, flavor, smell and are part of plants natural defense system.

Numerous fungal agents causing opportunistic infection have become serious threats to people`s life. Previous literature indicated that certain species of fungal agents have developed resistance to most antifungal preparations. (Fidel, 2002, Hassan *et al*,.2007 and Sakharkar and patil, 1998) unfortunately, the availability of this medication for the treatment of this disease is either expensive, out of reach of most patients or their use is associated with the undesirable side effects or both (Agassounon, 2011).

This necessitates the need to find novel and effective antifungal agents from plants natural product that are available, cheaper and affordable. Plant notably *Senna occidentalis,* and *Boswellia dalzielii*, have been documented to be effective against fungal species (Clark, 1981, Eseyin *et a*l., 2005; Etuk *et al.,* 2006 and Kunle 2000). The current work attempted to study the efficacy of some of these plant extracts in the treatment of some opportunistic fungal isolates specifically *Candida species* and *Aspergillus species*. The main aim of this work is to separate various fractions of the selected plants leaves extract using chromatographic techniques and to also assess the column chromatographic fraction of each of these plants for antifungal activities.

MATERIALS AND METHODS

Sample Collection

Collection of Plants Samples

The plants leaves of *Senna occidentalis and Boswellia dalzielii* were collected within Sokoto metropolis. The plants were taken to herbarium of the Botany unit of the Department of Biological science, Usmanu Danfodiyo University Sokoto for authentication. The voucher specimen of the plants were retained in the Departmental Herbarium with the voucher number UDUH/ANS/0023, and UDUH/ANS/0069 .The leaves collected were air dried and pulverized into fine power using pestle and mortar and stored in sealed containers until required.

Collection of Test Organisms

The strains of the organisms used were obtained as stock culture from mycology laboratory of the Department of Biological Sciences Usman Danfodiyo University Sokoto. The species of organisms include; *Candida albican, Candida pseudotropicalis, Aspergillus niger* and *Aspergillus flavus*.

Extraction Procedure

The extraction of the plants materials was carried out in accordance with the procedure of Abdullahi and Lawal(2010). Fifty grams (50g) each of the powdered plant material was soaked in 250ml of methanol and n- hexane.

The mixture was shaken daily for three (3) days at regular intervals, after which it was filtered using whatman no 11 filter paper. All the extracts were evaporated using rotary flash evaporator, and preserved in air tight bottle prior to the commencement of the analysis.The extracts were subjected to chromatographic studies and antifungal activity determination.

Chromatographic Techniques

Fractionation of the plants extract was carried out by activity guided fractionation. The procedure was carried out using ethyl acetate-hexane (20:80 v/v) using thin layer chromatography.

Column preparation

The column chromatographic fractionation was carried out on *Senna occidentalis* and *boswellia dalzielii* leaves extracts. the lower end of a glass column 10cm long and 1.5cm in internal diameter was plugged with glass wool. The plant material was poured on to the glasss wool and air bubbles release was trapped with the flat end of a packed rod.The column was packed with wet silica gel by pouring the silica gel into the column in a stepwises manner.the side of the column was taped gently with a glass rod for compaction of the particles. As the silica gel setteles, the column outlet was adjusted. Two grams (2g) of each samples was drawn into the adsorbent and elute with ethyl acetate-hexane. As the band reaches the bottom of the column, the layer was collected in the test tube and the test tube was change as the eluent change color. Ten fraction was obtained from methanolic extract and fifteen from n- hexane. (Nicolas, 2003).

Thin layer chromatography preparation

Thin layer chromatography (TLC) was used to separate the fraction that have the same spot on the chromotoplate. The chromatograms developed on the microscope slide, were dried and observed visually for the various leave compounds. The entire fractions that have the same spots were mixed together. Five fractions were obtained each from methanol and n-hexane leaves extracts.

Assessment of the column chromatographic fractions of the plants extracts for antifungal activities.

The antifungal activity of the column fraction of *Senna occidentalis and boswellia dalzielii* [methanol and n-hexane extracts] was carried out using agar incorporation method according to procedure of zacchino *et al;* [1999].5mls of column fraction was aseptically mixed with 15mls of Sabouraud dextrose agar. Then the media was poured aseptically in a sterile Petri dishes and allowed to solidify. Itraconazole positive control was measured from pulverized 500mg tablet in 5mls of sterile distilled water. After cooling and solidification of media, seeding was carried out by inoculation of all the fungal isolate in the middle of the petri dishes. Replicate for each fraction was made and incubated at ambient room temperature for 7-14 days. The zone of inhibition was measured.

RESULTS

Column chromatographic fractions of *Senna occidentalis* and *Boswellia dalzielii* leaves extract (methanol and n- hexane) was carried out. The column fractions revealed five fractions in each extract (n- hexane fractions 1to 5 and methanol fractions 1 to 5). The antifungal activity of column chromatographic fractions of n- hexane leaves extract of *Senna occidentalis* are indicated in table 1. The fractions were active against *C. albican, and C. pseudotropicalis* at concentration of 10, 20 and 30 mg/ml, and little effect against *A. flavus* with no activity against *A. niger*.

The results of analysis of variance conducted, revealed that there was no significant difference between plants tested against the fungal isolates

The result of the antifungal activity of column chromatographic fractions of methanolic extract of *Senna occidentalis* is presented in table 2. The results showed high antifungal activities against *C. albican, C. pseudotropicalis* at both concentrations of 10, 20, and 30mg/ml, with little effect against *A. niger* and *A. flavus* especially at concentration of 10mg/ml. The results of ANOVA conducted indicated that there was no significant difference between the tested plants against the organism.

The result of the antifungal activity of column fractions of n- hexane extract of *Boswellia dalzielii* is presented in table 3. The hexane fractions 1-5

showed highest activity against *C. albican* and *C. pseudotropicalis* at both the concentrations of the extract used, but with little activity against *A. flavus* and no activity against *A. niger*. Analysis of varience conducted on the results revealed that there is no significant difference between the tested plants against organisms.

Table 4 showed the antifungal activity of column chromatographic fraction of methanol leaves extract of *Boswellia dalzielii*. The results indicated high activity of the fractions against *C. albican* and *C. pseudotropicalis* at various concentrations of the extracts. The results also showed high activity against A. niger at 20 and 30 mg/ml concentration, with less activity against *A. Flavus*. Analysis of varience was carried out and reveals that there is no significant difference between the plants tested against organisms.

Table 1: Antifungal activity of column chromatographic fractions of *S occidentalis* methanolic and n-hexane extract

Plant leaves	Organic solvent	Extracts conc.	Growth (mm)		
	Extract	(mg/ml)	*C.albican*	*C.pseudotropicalis*	*A.niger*	*A.flavus*
	Hexl	10	8	7	2	3
		20	8	6	2	5
		30	8	7	5	5
	Hex2	10	7	5	3	2
		20	8	7	3	5
		30	8	6	2	5
	Hex3	10	7	7	3	3
		20	8	7	3	5
		30	8	7	3	5
	Hex4	10	6	5	2	5
		20	7	6	2	5
		30	7	6	3	5

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S.occidentalis		Hex5		10		6	7		3	5
				20		6	7		3	3
				30		6	7		3	2

Key
Hex1-5= n- hexane fraction of Senna occidentalis.
Values > 4mm shows no zone of inhibition

Table 2: Antifungal activity of column chromatographic fraction of *Senna occidentalis* methanolic
Leaves ext

Plant leaves		Organic solvent	Extracts conc.		Growth (mm)			
		Extract	(mg/ml)		*C.albican*	*C.pseudotropicalis*	*A.niger*	*A.flavus*
Met1		10			18	20	*7*	*6*
		20 30			27 30	20 30	10 15	8 9
Met2		10			19	10	7	6
		20			30	20	10	9
S.occidentalis		30			32	27	12	9
Met3		10			18	11	6	5
		20			19	16	8	6
		30			32	30	13	9

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Met4		10		18		20		5	5
		20		27		19		7	9
		30		30		27		12	9
Met5		10		17		19		4	6
		20		29		30		11	7
		30		32		30		15	8

Key
Met1-5, methanol fraction 1-5 of *Senna occidentalis* leaves extract.
Values > 4mm shows no zone of inhibition

Table 3: Antifungal activity of column chromatographic fractions of *Boswellia dalzielii* n-hexane extract

Plant leaves		Organic solvent		Extracts conc.		Growth (mm)					
		Extract.		(mg/ml)		*C.albican*	organisms *C.pseudotropicalis*	*A.niger*		*A.flavus*	
		Hex1		10		8	8	2		3	
				20		8	7	2		4	
				30		8	7	5		5	
		Hex2		10		7	5	2		2	
				20		8	7	3		5	
				30		8	6	5		5	
		Hex3		10		6	7	2		5	
				20		7	7	2		2	
				30		7	7	2		3	
		Hex4		10		6	5	3		5	
				20		8	6	3		5	
				30		7	6	3		5	

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		Hex5		10		7	6	2		5	
				20		7	7	3		4	
				30		7	6	5		4	

Hex1-5= N-hexane fraction 1-5, of *Boswella dalzielii* leaves extracts
Values > 4mm shows no zone of inhibition

Table 4: Antifungal activity of column chromatographic fraction of Boswellia dalzielii methanolic leaves extract.

Plant leaves	Organic solvent	Extracts conc.	Growth (mm)			
	Extract.	(mg/ml)		*C.albican*	*C.pseudotropicalis*	*A.niger*	*A.flavus*
		10		22	7	7	6
		20		22	11	10	8
		30		30	13	11	9
		10		10	18	7	6
		20		20	18	8	7
		30		27	32	10	7
		10		10	17	7	5
		20		20	19	9	6
		30		30	21	10	8
		10		10	9	6	5
		20		12	11	8	9

		30		14	12	13		9
.		10		17	19	4		6
		20		29	30	11		7
		30		32	30	15		8

Key
Met1-5= Methanol fraction 1-5 of *Boswelladalzielii* leaves extracts
Values > 4mm shows no zone of inhibition

Discussion

Fractionation of the methanol and n- hexane extracts of *Senna occidentalis* and *Boswellia dalzielii* are showed in tables 1, 2, 3 and 4, where the methanol fractions 2 and 5 of of *Senna occidentalis*, had the highest antifungal activity with the diameter zone of inhition of (32.0 mm). The n-hexane fraction 1 of the same plant showed the diameter zone of inhibition of 8.0mm. The methanol fractions 5 of *Boswellia dalzielii* leaves extracts also recorded highest activity, with the diameter zone of inhibition of 32.0mm. The n- hexane fraction 1 recorded the diameter zone of inhibition of 8.0mm. The findings of this work totally contradicts the work of Hassan et al., (2007), where the hexane fractions of 2 and 3 of the stemp bark extract of Ficus sycomorus L. were found to be significantly active at concentrations of 0.31 to 5.63 mg/ml^{-1} on *Microsporum gypseum*, ***Aspergillus niger***, ***Aspergillus flavus*** and *Candida albicans*. This difference may be due to the fact of using different plants and also plants parts. Present results are also comparable to those of Subramanian ***et al***. (2006) who showed **ethanolic extracts** of ***Aloe vera*** gel, although a different plant family, to have activity against most of the pathogenic fungi at very low doses.

The active components of the solvents with each of fraction inhibit the growth of the isolates (especially *candida albican and candida pseudotropicalis*) but with low effect on the growth of *Aspergillus niger and aspergillus flavus* at the concentration employed, it could be possible that, the concentration employed is low to inhibit the growth of these isolate. These showed the ability of methanol and n- hexane to effectively

extract antifungal components. This justified it is use in the fractionation of medicinal plants used in the treatment of fungal diseases, the result obtained here is comparable to that of (Adelakun *et al*., 2001; Jain *et al*., 1998; Saganuwan, 2006 and Abo, 1998).

Conclusion

This research scientifically justifies the use of leaves extracts of *Senna occidentalis* and *Boswellia dalzielii* for antifungal therapy, thus presenting a potentially new cheap source of potent antifungal agent.

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