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ANTIFUNGAL ACTIVITY OF POLAR, NONPOLAR AND AQUEOUS EXTRACTS OF THE LEAVES OF *Ziziphus mucranata* AGAINST *Aspergillus* specie

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ABSTRACT
The current research investigates the antifungal properties of crude extracts (i.e aqueous, methanol, and dichloromethane) of the leaves of Ziziphus mucranata. The plant leaves were procured and identified by the taxonomist at the UDUS sokoto, immediately washed to remove debris, dried under the shade for two weeks, ground into powder. Fifty grams of powdered material was extracted individually with 200ml of different solvents (Dichloromethane (DCM), and Methanol) with the exception of aqueous extract which was extracted with 400ml for 48 hours in a laboratory through cold extraction method, then filtered, concentrated and kept for antifungal analysis. The media used were potato dextrose agar (PDA), prepared according to the manufacturer's instruction. Qualitative testing for antifungal activity against (Aspergillus flavus and Aspergillus niger) was determined by agar well method. The results obtained showed that the methanol extract has the highest zone of inhibition on Aspergillus flavus, followed by DCM extract, while Aq extract recorded the least activity. However, methanol extracts maintained highest activity on Aspergillus niger while Aq and DCM extracts shared the least activities. There is need to call for thorough investigation and identification of the active compounds responsible for these and other unexplored activities.
Keywords: Antifungal activity, Fungal isolates, Extracts and ziziphus mucranata.

INTRODUCTION

Plants has provided man with all his needs in terms of food, shelter and herbal medicines, among other which formed the basis of sophisticated traditional medicine systems that have been in existence for thousands of years and continue to provide mankind with new remedies. A medicinal plant is used in order to relieve, prevent or cure a disease or to alter physiological and pathological process or their precursors (Arias, 1999)

The Knowledge of medicinal plants has usually resulted from trial and error methods and often based on speculation and superstition (Hamayun et al, 2006). This has contributed to humanity's health care, source of livelihood cultural traditions, and financial gains among others constrained by procedures such as classification, identification and characterization (Hamilton, 2004). About 80% of the world's population still relies upon plants for primary health care, even today in western medicine, and despite progress in synthetic chemistry, some 25% of prescription medicines are still derived either directly or indirectly from plants due to its constituents which are both beneficial and harmful to mankind (Farnsworth and Soejarto, 1991). As a result of this, there is need to carry out scientific investigations on the plant which could provides information with respect to the presence of some phytochemical compounds that includes: Alkaloid; Tannins, Saponins, Flavonoids, cardiac glycosides, and many others and their physical effects of (Akindahunsi and Salwau, 2005).

The plant *Ziziphus mucranata* (Rhamnaceae) commonly known as Buffalo thorn, is native to Northern Nigeria; the plant decoction is used traditionally in the treatment of diabetes mellitus among the rural population of Northern Nigeria (Etuk *et al*. 2010). The fruits are edible and nutritious though not very tasty and somewhat mealy. In times of scarcity, they can be eaten fresh, dried or made into a meal or porridge. The fruits quencher and is fermented to prepare a beer (Ellis, 2003). In East Africa, roots of *Ziziphus mucranata* are used for treating snake bites (Mazibuko, 2007). However, scientific reports on any pharmacological activity from this plant are scanty in literature. Hence, this study is designed to evaluate the antifungal screening of the leaves of *Ziziphus mucranata*

MATERIALS AND METHODS

The leaves sample of *Ziziphus mucranata* (Buffalo thorn) was freshly collected in the month of January, 2016 from Central Market, Sokoto State, Nigeria. The plant leaves were collected and identified at the Department of Botany, Usmanu Danfodiyo University Sokoto, Nigeria. The leaves were immediately washed with distilled water to remove debris, dried under the shade for about two weeks and then grinded into fine powder using a kitchen blender and stored in air tight bottle kept in desiccator till use.

Extraction of Plant Materials

Fifty grams powdered material was extracted individually with 200ml of different solvents (Dichloromethane (DCM), and Methanol) with the exception of aqueous extract which was extracted with 400ml for 48 hours in a laboratory through cold extraction method, then filtered, concentrated and kept for antifungal analysis. The percentage yield was recorded.

Thin Layer Chromatography

TLC was carried out on the leaves extract using commercially prepared silica gel coated TLC plates (5 to $20cm^3$). The extracts were dissolved in a little individual solvents (Dichloro methane, Methanol and Aqueous) and the solution of each extract spotted on the line drawn 2cm near or from the bottom edge end of the plate using capillary tube. The chromatogram of MeOH and DCM extracts was developed with MeOH – Ethyl acetate – DCM (1:2:3) solvents while chromatogram of aqueous extract was developed using MeOH–H_2O (1:1) solvents. The dried chromatograms were visualized by spraying 5% H_2SO4 solution. The R_f value of each band was calculated.

Antifungal Activity

Preparation of Media

The media used were potato dextrose Agar (PDA). It was prepared according to the manufacturers instruction, thirty nine (39g) was weighted by using a digital weighing balance and dispersed into 1500ml conical flask that contained 1000ml of distilled water, it was then plugged with cotton wool and wrapped with aluminum foil, heated for complete dissolution. The dissolved media was then sterilized by autoclaving at 121^0c for 15 minutes to ensure sterilization. The media was allowed to cool to 45minutes at room temperature. It was then poured into sterilized petri-dishes 15-20mls, allowed to solidify for 24hours, before inoculation of the sample.

Collection of the Sample isolates

Samples were collected from the slanted bottles, preserved in mycology laboratory of Biological Sciences Department, Usmanu Danfodiyo University. They were *Aspergillus niger* and *Aspergillus flavus*. Culture obtained was sub cultured by using sterile cork borers to cut out 2mm disc from advancing region of the cultured colonies. Isolates were microscopically examined with the view to re-identify the organisms.

Microscopic examination was carried out in accordance with Mackie and McCartney to re-confirm the isolates. Slide mounted made with lacto phenol cotton blue to improve visualization and viewed under the microscope at x40 magnification.

Antifungal activities

Qualitative testing for antifungal activity of two (2) fungal isolates was determined by agar well method which was carried out according to the procedure of Collins *et al.,* (1995).

Thirty nine (39g) of potato Dextrose Agar was prepared and allowed to solidify as the medium for testing the isolates and cork borer of 12mm in diameter was also used. Four (4) wells were made in each plates of potato Dextrose Agar with a sterilize 12mm in diameter cork borer plates, the wells were aseptically filled by using different syringe of different extracts. The plates were left standing in a work bench for two (2) minutes to allowed the extracts to settled, each plates was then seeded with a test organisms at the centre of the plate. Antifungal extracts was used as positive control while 30% methanol in water was used as negative control. The plates were then incubated at room as negative control. The plates were then incubated at room temperature for two (2) weeks, evidence of growth around the well indicated antifungal activity against the fungal pathogens the diameter of the growth were measured and value for each test organism was recorded (Olukoya *et al*, 1993).

RESULTS AND DISCUSSIONS

Table 1: TLC analysis of three (3) solvent extracts of the leaves of *Ziziphus mucranata*

Extract	Solvent system	Number of components	Distance of spot(cm)	Solvent front (cm)	RF value
Aqueous	MeOH:H2O(1:1) =(5cm^3:5cm^3)	2	6.30cm	7.10cm	0.89
			5.70cm	7.10cm	0.80
Methanol	MeOH:Ethylacetate:DCM(1:2:3) =(2cm^3:4cm^3:6cm^3)	10	7.70cm	8.00cm	0.96
			7.30cm	8.00cm	0.91
			6.00cm	8.00cm	0.75
			5.60cm	8.00cm	0.70
			5.30cm	8.00cm	0.66
			4.50cm	8.00cm	0.56
			3.10cm	8.00cm	0.39
			2.10cm	8.00cm	0.26
			1.1cm	8.00cm	0.14
			0.8cm	8.00cm	0.10
DCM extract	MeOH:Ethyl acetate: DCM(1:2:3) =(2cm^3:4cm^3:6cm^3)	5	7.50cm	8.00cm	0.94
			7.00cm	8.00cm	0.88
			5.70cm	8.00cm	0.71
			5.30cm	8.00cm	0.66
			4.80cm	8.00cm	0.60

Thin Layer Chromatography was used to identify numbers of components presence in the sample extracts, in which two (2) spots were detected in the chromatogram clearly for aqueous extract using methanol (MeOH): water (H_2O) in the ratio of 5cm^3:5cm^{3}as solvent system and the R_f values were found to be 0.89 and 0.80 respectively. Furthermore, ten (10) spots were detected in methanol extract using methanol (MeOH): Ethyl acetate: Dichloromethane (DCM) in the ratio of 2cm^3:4cm^3:2cm^{3}as solvent system and the R_f values were found to be 0.96, 0.91, 0.75, 0.70, 0.66, 0.56, 0.39, 0.26, 0.14 and 0.10 respectively.

Dichloromethane extract on the other hand was detected to possess five (5) spots using methanol (MeOH): Ethyl acetate: Dichloromethane (DCM) in the ratio of 2cm^3:4cm^3:2cm^{3}as solvent system and the R_f values were found to be 0.94, 0.88, 0.71, 0.66 and 0.60 respectively.

The result of Thin Layer Chromatography (TLC) obtained in this investigation shown above indicates that Methanol extract has highest distance of spot (cm) which ranged between 7.30cm to 0.8cm with 0.10 to 0.96 as R_f value. The least extract with distance spot was Aqueous with 5.70cm to 6.30cm and 0.89 R_f value. All the extracts have 8.00cm solvent front except aqueous which possessed only 7.10cm as presented in table 1 above.

Table 2: Zones of inhibition of *Aspergillus flavus and A. niger* (centimeter)

Treatment	***A. flavus***	***A. niger***
Aqueous extract	1.70cm	1.30cm
Methanol extract	4.80cm	3.00cm
Dichloromethane extract	4.50cm	1.30cm

Note: The diameter of the agar well is 0.60cm, therefore any well greater than 0.60cm shows activity.

The percentage yield (%) of the extracts was quantified, whereby Aq, MeOH, and DCM extracts possessed 0.92%, 2.20%, and 1.65% respectively. They were then subjected to anti-fungal activity to ascertain their efficiency. The species of fungi associated with tomatoes were isolated as *Aspergillus flavus*, *Aspergillus niger*, *Yeast Spp.* and *Fusarium* yielded 36.5%, 14.5%, 16.4% and 32.7% respectively. Two of the species were selected and treated with different extracts for the zone of inhibition (centimeter). The aqueous extract showed activity on *Aspergillus flavus* and *Aspergillus niger* at 20mg/ml concentration to give 1.70cm and 1.30cm respectively. The methanol extract on the other hand showed activity on *Aspergillus flavus* and *Aspergillus niger* at 20mg/ml concentration to yield 4.80cm and 3.00cm respectively, and finally dichloromethane extract showed activity on *Aspergillus flavus* and *Aspergillus niger* at 20mg/ml concentration to obtain 4.50cm and 1.30cm respectively.

The results obtained show that the methanol extract has the highest zone of inhibition on *Aspergillus flavus*, followed by DCM extract, while Aq extract recorded the least activity. However, methanol extract maintained highest activity on *Aspergillus niger*, while Aq and DCM extracts shared the least activities as indicated table 2.

Table 3: Antifungal sensitivity profile

Species	Methanol	Aqueous	Dichloromethane
Aspergillus flavus	R	S	R
Aspergillus niger	I	S	S

KEY POINTS: S = susceptible, I = Intermediate and R = Resistant.

The Sensitivity profile conducted using different extracts indicates that, Methanol and Dichloromethane have resistant to *A. flavus*, while aqueous and Dichloromethane have susceptibility to *A. niger* and methanol has intermediate action to *A. niger a*s presented in table 3 above (susceptible ≥ 1.00cm, intermediate ≥ 2.90cm and above 3.00 is considered resistant) Pfaller *et al*, 2009. This shows that *Aspergillus flavus* was resistant to all extracts except Aq extract that shows susceptibility, while *Aspergillus niger* was susceptible to all extracts except methanol extract that shows intermediate.

CONCLUSION

Leaves of *Ziziphus mucronata* reveal some degree of anti-fungal activities against *Aspergillus flavus* and *Aspergillus niger* and these activities may be traced to either alkaloid steroids. These indicate that most of medicinal plants used in ethnomedicine are potentially useful pharmacological and nutraceutical in the treatment of some pathogenic microorganisms. This research may contribute to the clear understanding of antifungal activities of the plant. On account of this vital information therefore, there is need to call for thorough investigation to isolate and characterize the active compounds responsible for these and other unexplored activities.