

Original Research Paper

Antifungal Potential of *Leptadenia Hastata* Against Some Pathogenic Fungi

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Abstract: The objective of this study was to evaluate if the plant extract could provide antifungal potential against some pathogenic fungi. Extract of Dichloromethane solvents was used for disc diffusion assay. The inhibitory concentration of the extract was performed by broth dilution method and zone of inhibition was studied by disc diffusion method at the concentration of 25, 50, 100, 250, 500 and 1000ppm in DMSO. Fluconazole was used as the reference control for antifungal study. The extract showed maximum inhibition potential of zone of inhibition against most of the pathogen (*Aspergillus niger*, *Aspergillus flavus*, *Candida tropicalis* and *Fusarium oxysporium*) used at concentration 25ppm to 250ppm with zone of inhibition (3.45±0, 3.33±0.12, 3.07±0.05 and 2.97±0.10mm respectively). The extract showed minimum inhibition potential against *Fusarium oxysporium* in all the concentration when compared with the control as well as to the other pathogens. *Aspergillus niger* and *Aspergillus flavus* was found to be more sensitive to Dichloromethane leaf extract followed by *Candida tropicalis* and lastly *Fusarium oxysporium*. The Present study indicates the potential usefulness of Dichloromethane leaf extract of *Leptadenia hastata* as antifungal agent.

Keywords: *Aspergillus Niger*, *Aspergillus Flavus*, *Candida Tropicalis*, *Fusarium Oxysporium*, *Leptadenia Hastata*, Fluconazole

Introduction

Leptadenia hastata (pers.) Decne belonging to family Asclepiadaceae is one of such medicinal plants, locally known as yadiya in Nigeria and Niger, *hagalhadjar* (Arabic) in Chad, *hayla* (Kusume) Ethiopia, *ekamongo* (Turkana) in Kenya, *lolongo* (Moore) in Burkina Faso, *tarhat* or *darhat* (Wolof), *busumba amata* (Jola) in Senegal and *nzongnè* (Bambara) in Mali. (Freiberger *et al.*, 1998; Aberkane *et al.*, 2002; Sena *et al.*, 1998). It is an erect, ascending, shrub up to 1.5-3m high with green stem and pale green alternating branches with watery sap. The plant is commonly used in traditional system of medicine for relieving pain and inflammation, as well as in a number of metabolic disorders such as diabetes and obesity.

The plant represents a rich source of antimicrobial agents. It is used medicinally in different countries and it is a source of many potent and powerful drugs. A wide range of medicinal plant parts extract are used as raw drugs and are said to possess varied medicinal

properties. The different parts used include root, stem-bark and leaves. Some of these raw drugs are collected in small quantities by the local communities and folk healers for local use, many other raw drugs are collected in larger quantities and traded in the market as the raw material for many herbal industries (Ambikapathy *et al.*, 2011).

Considering the vast potentiality of this plant *Leptadenia hastata* as sources for antimicrobial drugs with reference to antifungal agents. The present study was designed to evaluate the invitro anti-fungal potential of leave extract against four different fungal species, *Aspergillus niger*, *Aspergillus flavin*, *Candida tropicalis* and *Fusarium oxysporium*.

Materials and Method

Plants were collected from Michika Local Government Adamawa state Nigeria. Identification of plants was done through herbarium available in the Ahmadu bello University Zaria

Extract Preparation

The leaves were washed with distilled water to remove the soil and dust particles, they were thoroughly air dried and powdered using laboratory grinder machine (FGR-350, Quest Scientific) extraction using dichloromethane by placing 150g of the powdered samples into an Erlenmeyer flask and dichloromethane three times the weight of the extracts was added, the solution was covered and shaken at an interval of an hour and then allowed at room temperature to stand for 7days, the mixture were then filtered using Whatman filter paper No.4 and the solvent was evaporated using a rotary evaporator (Heidolph Laborato 400) under reduced pressure below 50EC. It was then stored under a frozen condition until required. 2 g of the extract was weighed and dissolved in 20 mL of Dimethyl sulfoxide (DMSO) to make a stock concentration of 100mg/ml from which the various concentrations used were calculated. Fluconazole common name Diflucan (Pfizer Inc New York, NY) was used as reference standard for antifungal studies.

Antifungal Potential

The antifungal potential of the plant extract was performed by agar disc diffusion method. Dimethyl sulfoxide DMSO was used as a negative control and Fluconazole (Diflucan) was used as a positive control. The plates were incubated at 37°C. The antimicrobial activity was taken on the basis of diameter of zone of inhibition in triplicate, which was measured before and after 5days of incubation and the mean of three readings is presented. The presence of inhibition of the treated fungus was calculated using positive control as standard (100% inhibition). (Cruickshank, 1975; Karwa *et al.*, 1997; Aboh *et al.*, 2014).

The plant extract and the standard antifungal agents were dissolved in DMSO, 100% biologically inert substances, with the disc diameter of 6mm. The extracts were separately dissolved in dimethyl sulphoxide. This (DMSO) solvent served as reference control for the antifungal study. The solvent control (DMSO) was also maintained throughout the experiment. Potato dextrose agar media was used for the antifungal study. The molten media was then inoculated with 200µl of the inoculums (1×10^8 Cfu) and poured into the sterile Petri plates. The disc was saturated with 20µl of the extracts separately, allowed to dry and was introduced on the upper layer of the seeded agar plate. The plates were incubated at 28°C and the zone of inhibition was measured every after 24 h for five days.

Fungal Preparation

The fungi were standardized by inoculating sterile normal saline solution with a 48 h pure culture by adjustment of turbidity to match 0.5 McFarland standard.

Standardization of the microorganisms included harvesting fungal spores from a 7 days old culture on SDA slant. Ten milliliters of sterile normal saline containing 3% w/v Tween 80 was used to disperse the spores with the aid of sterilized glass beads (Olowosulu *et al.*, 2005). Standardization of the spore suspension to 1.0×10^6 spores/mL was achieved with a UV spectrophotometer (Spectronic 20D; Milton Roy Company, Pacisa, Madrid, Spain) at 530 nm (OD at 530) of the suspensions and adjusted to a transmittance of 70-72%. The plates were incubated at 37°C for 24h (Aberkane *et al.*, 2002).

Statistical Analysis

Values were expressed as Mean±standard deviation for three determinations of each experiment. The analysis was done using the software-SPSS one way ANOVA.

Results and Discussion

Results

The result as presented in Table 1 shows that the plant extract was effective in significantly reducing the growth of the pathogen as compared to the control drug. The dichloromethane extract of *Leptadenia hastata* inhibits the growth of *Aspergillus niger*, *Aspergillus flavus*, *Candida tropicalis* and *Fusarium oxysporium* in culture medium. The zone of inhibition is significant in all the solvent extract concentration and lower in 500, 1000ppm. *Aspergillus niger* shows a higher inhibition compared to the control as well as at different treatment days at the same concentration on each fungus at 50 and 100ppm, the zone of inhibition were 3.45 ± 0.05 and 3.55 ± 0.05 mm and for 25, 250 and 500ppm was 3.15 ± 0.48 , 2.98 ± 0.04 and 2.38 ± 0.10 mm respectively.

The growth of these fungi *Aspergillus flavus* was inhibited at the 5th day with zone of inhibition of 3.10 ± 0.06 mm at 25ppm, 3.22 ± 0.08 mm, at 50ppm and 100ppm at 3.33 ± 0.12 mm, followed by 3.10 ± 0.06 mm at 250ppm. Table 1 shows the inhibitory effect on growth of these fungi these increases with the increase in days and concentrations of the extract.

Candida tropicalis, observation reveals that growth is more inhibited at the 5th day when compared with the others, at 25ppm the zone of inhibition was 2.98 ± 0.08 mm, 50ppm it was 3.00 ± 0.06 mm, while at 100ppm it was 3.07 ± 0.05 mm higher when compared to the different treatment days at the same concentration on each fungus as well as the control. However, inhibition increases with the increase in concentrations.

The growth of *Fusarium oxysporium* was observed to have been inhibited with increase in concentration too. The rate of inhibition at 25pp was 2.85 ± 0.14 , 50ppm was 2.90 ± 0.11 mm, while 100ppm was observed to be the highest by 2.97 ± 0.10 mm.

Table 1: Effect of dichloromethane leaf extract of *Leptadenia hastata* on Fungi

Organisms	Day	Concentration (ppm)						
		Control	25	50	100	250	500	1000
<i>Aspergillus niger</i>	1	0.86±0.05	0.78±0.12	0.80±0.09 ^d	0.63 ± 0.15	0.95±0.05 ^{bd}	0.47±0.12	0.18±0.08
	2	1.24±0.08	1.24±0.08	1.63±0.08 ^b	1.52 ± 0.10 ^d	1.60±0.14 ^d	0.80±0.08 ^d	0.45±0.10
	3	1.93±0.05	1.65±0.08	1.78±0.12	1.75 ± 0.05	1.45±0.08	0.75±0.10	0.48±0.07
	4	3.06±0.13	2.80±0.19	2.73±0.08	2.70 ± 0.06	2.50±0.06	1.78±0.12	0.88±0.08
	5	3.45±0.05	3.15±0.48 ^{ad}	3.45±0.05 ^{ad}	3.55 ± 0.05 ^{abd}	2.98±0.04 ^a	2.38±0.10 ^a	1.85±0.05 ^a
<i>Aspergillus flavin</i>	1	0.86±0.06	0.48±0.16	0.75±0.12	0.70 ± 0.00 ^d	0.52±0.15	0.50±0.20 ^d	0.23±0.12
	2	1.80±0.15	1.80±0.20 ^d	1.55±0.08	1.32±0.08	1.23±0.12	0.75±0.05	0.48±0.15
	3	2.10±0.09	1.80±0.09	1.73±0.10	1.70±0.16	1.42±0.31	0.82±0.21	0.37±0.14
	4	3.02±0.15	2.88±0.12 ^d	2.87±0.15 ^d	2.87±0.05	2.48±0.12	2.18±0.15	1.97±0.08 ^{ad}
	5	3.22±0.10	3.10±0.06 ^a	3.22±0.08 ^a	3.33±0.12 ^{ab}	3.10± 0.06 ^{ad}	2.90±0.09 ^{ad}	1.85±0.10
<i>Candida tropicalis</i>	1	1.05±0.06	1.27±0.37 ^{bd}	0.53±0.12	0.58±0.08	0.47±0.10	0.38±0.12	0.25±0.16 ^d
	2	1.85±0.06	1.67±0.10	1.73±0.08 ^d	1.30±0.09	1.23±0.10	0.78±0.08	0.53±0.05 ^d
	3	1.90±0.09	1.93±0.08 ^d	2.02±0.14 ^d	2.13±0.10 ^d	2.20±0.13 ^{bd}	1.92±0.08	1.80±0.18
	4	2.78±0.08	2.53±0.08	2.72±0.08	2.83±0.08	2.93±0.08 ^{abd}	2.28±0.21	1.95±0.10 ^a
	5	3.23±0.12	2.98±0.08 ^a	3.00±0.06 ^a	3.07±0.05 ^a	2.92±0.08	2.53±0.08 ^a	1.93±0.09 ^d
<i>Fusarium oxysporium</i>	1	0.70±0.09	0.45±0.16	0.43±0.19	0.57±0.12	0.42±0.17	0.37±0.16	0.18±0.08
	2	1.60±0.09	1.42±0.15	1.53±0.12	1.30±0.06	1.08±0.08	0.62±0.10	0.42±0.16
	3	2.02±0.08	1.93±0.10 ^d	2.02±0.08 ^d	2.07±0.14	2.02±0.16 ^d	2.18±0.15 ^{bd}	1.92±0.08 ^d
	4	2.82±0.08	2.50±0.14	2.38±0.25	2.72±0.15	2.77±0.12 ^a	2.48±0.08 ^{ad}	1.88±0.08 ^a
	5	3.23±0.13	2.85±0.14 ^a	2.90±0.11 ^a	2.97±0.10 ^a	2.62±0.08	2.32±0.16	1.43±0.12

Values are Mean±SD for six determinations

^aSignificantly (p<0.05) higher compared to different treatment days at the same concentration on each fungus

^bSignificantly (p<0.05) higher compared to the control on each fungus in each row

^dSignificantly (p<0.05) higher compared to other fungi on the same day on each concentration

Discussion

The study revealed the antifungal activity of the extract against the tested species of microorganisms between concentration ranges for 25 to 1000ppm. The results of this study revealed that the extracts possess antifungal activity in a concentration dependent manner against the test organisms and were comparable with the standard drug.

The inhibition yielded at concentration 25-250ppm was found to be active against all tested strains under study. However, the extract showed higher significant antifungal activity against *Aspergillus flavus* and *Aspergillus niger* over the other pathogens. Though the extract showed moderate activity at all concentration on the fungal species, the potency exhibited less significant activity against *Candida tropicalis* as well as *Fusarium oxysporium* strains. Thus, the antimicrobial activities of plant related have also been found registered in various literature (Satish *et al.*, 2007; Bhardwaj, 2012., Gujar and Taknkar, 2012; Ilyas *et al.*, 1997; Dubey and Kumar, 2003; Dubey *et al.*, 2009) found almost similar effect of *Leptadenia hastata* dichloromethane extract on growth of some other fungi's.

The result of this study indicating that differential activities of the plant extracts on the growth of different fungi, because many of these extract concentration has shown significant and aggressively inhibition against the growth of some of this tested fungus.

The antifungal activities of this medicinal plants are attributed due to the presence of flavonoids and tannins. (Barnabas and Nagarajan, 1988). The presence of this metabolite in the leaf extract confirm its potential against the selected pathogens. This shown the significant and aggressive inhibition which was observed against the pathogen as it suppressed the growth of all the pathogenic fungi. Thus, the potential usefulness of dichloromethane leaf extract of *Leptadenia hastata* in the treatment of various pathogenic diseases, as well as helping in the discovery of new chemical classes for antifungal drugs that could serve as selective agents for the maintenance of human health should be considered.

Conclusion

The outcome of this study supports the traditional medicine uses of different plant extract concentration in the treatment of different infections caused by pathogenic fungi, indicating the usefulness of dichloromethane leaf extract of *Leptadenia hastata* as antifungal agents.

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Author's Contribution

Isaac John Umaru: Design of experiment, data analysis, discussion and write of the manuscripts.

Fasihuddin A Badruddin: Coordinator for research, design research experiment and supervision of work.

Henry Y. Wakawa: Proof read of the manuscripts

Hauwa A. Umaru and Kerenhappuch I Umaru: Coordinator, Sample collection, preparation and data analysis.

Ethics

This article is original and contains unpublished material. The corresponding author confirms no conflict of interest and all other authors have read and approved the manuscript. No ethical issues involved.

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