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FUNGAL INFECTION AND THE ROLE OF *LEPTADENIA HASTATA (PER) DECNE.*

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ABSTRACT

Fungal infections (mycoses) are likely to occur more frequently as ever-increasingly sophisticated healthcare systems create greater risk factors, the limited access to more powerful diagnostic tools and agents for fungal infection, there is a paucity of data and drugs regarding the burden of fungal infections in the world. The main objective of this study was thus to study the fungal infection and the role of *Leptadenia hastata* Stem-bark overcome the burden of severe fungal infections in the world thus establishing reasonable agents to fight the incidence and prevalence's. Material and method; the fungal activities of the extract at various concentration were performed by agar diffusion method, DMSO as negative control while Fluconazole as positive control for five days. One-way ANOVA was used to determine the statistical difference between three mean inhibition value at significance $P<0.005$. Result: The concentration of extract at days four and five, 25ppm, 50ppm and 100ppm showed the highest activities against Aspergillus flavus, Aspergillus niger, Candida tropicallis, and Fusarium oxysporium was very significant when compared with the control drug. Conclusion; The results of investigation indicate significant inhibition to reduce the burden of human fungal infections in the world.

KEYWORDS: Aspergillus flavus, Aspergillus niger, Candida tropicallis, Fusarium oxyporium, Leptadenia Hastate.

INTRODUCTION

Nearly a billion people are estimated to have skin, nail and hair fungal infections, many 10's of millions mucosal candidiasis and more than 150 million people have serious fungal diseases, which have a major impact on their lives and can be fatal. However, severity ranges from asymptomatic-mild mucocutaneous infections to potentially life-threatening systemic infections. Moreover, mortality associated with fungal disease at greater than 1.6 million is similar to that of tuberculosis and greater than three-fold more than malaria. Socioeconomic, geo ecological characteristics and the increasing number of at-risk populations are the main determinants of variations on incidence and prevalence of fungal disease across the world. HIV/AIDS pandemic, tuberculosis, chronic obstructive pulmonary disease (COPD), asthma and the increasing incidence of cancers are the major drivers of fungal infections in both developed and developing countries globally.[1]

However, fungal infections only represent a secondary priority at a national level in some African countries when compared with the developed countries.[2] The fungal burden of serious fungal infections may be largely underestimated. This is mainly due to the limited access to adequate and reliable diagnostic tests, but also to erroneous presumptive diagnoses in favour of other prevalent infections, as reported for histoplasmosis and tuberculosis in Cameroon.[3]

Over the last decades, the incidence of fungal infections in human beings has considerably increased and epidemiologic change regarding the fungal infections has been reported in many hospitals around the world. The species causing infection are more diverse. Several have become prevalent in some hospitals due to the intrinsic resistance against widespread antifungal agents.[4]

Traditionally Candida and Aspergillus species are accounted for the majority of infections. Candidemia is the fourth leading cause of blood-stream infections and carries 35-55% mortality. The incidence of mould infections has also increased in the recent past, especially infectious caused by Aspergillus spp. where the mortality rate crosses 50% in such patients. Mucormycosis is a threat in uncontrolled diabetes in developing countries.[5]

Candidiasis among others is extremely common. Up to 75 per cent of women have experienced thrush at some time and about 102 per cent of men attending

genitourinary balanitis (infection of the head of the penis) caused by Candida.[6] Oral candidiasis is common in infants exposed to the organism during passage down the birth canal or during breastfeeding.

Fungal diseases kill more than 1.5 million and affect over a billion people. However, they are still a neglected topic by public health authorities even though most deaths from fungal diseases are avoidable. Early accurate diagnosis allows prompt antifungal therapy; however, this is often delayed or unavailable leading to death, serious chronic illness or blindness.

The main objective of this study was to look at the role of *Leptadenia hastata (per) decne.* on the burden of some fungal infections in reducing the incidence and prevalence. E.g. *Aspergillus niger, Aspergillus Flavin, Candida tropicalis and Fusarium oxysporium.*

Leptadenia hastata is typically grown in tropical dry lands in sandy soil. Wild foods like this plant provide food security during seasonal changes and are used medicinally in many areas. The plant belongs to many of the root-type famine food plants, they are drought tolerant and can stay in the soil intact for a long time. Therefore, they can be collected when the need is greatest. Most of the leafy-type famine-food plants are locally referred to and classified as 'weeds', sprouting and flourishing after rains. There are two main periods of maximum consumption of the leaves and tender parts of such famine-food plants. The first period is while farmers are waiting for the upcoming crop harvest and, the second main period is when they run out of food stocks from the previous harvest and are hence facing a food shortage. People try, whenever possible to add famine-food to local staple foods or to mix it with other foodstuff to mask the often-offensive nature of the food and to reduce any characteristic and unpleasant side effects. The vernacular names for *Leptadenia hastata* include; hagalhadjar in Arabic and in Chad, yadiya in Hausa in Nigeria and Niger, while in kusume hayla in Ethiopia, ekamongo from Turkana in Kenya, Moore people call it lolongo in Burkina Faso, tarhat or darhat from Wolof in busumba, amata from Jola in Senegal, and Bambara's call it nzongne in Mali.[7] It is a very drought-resistant plant and can grow with 100 to 450 mm/year. This plant can tolerate high pH and high exchangeable sodium and potassium within the environment.[8] The leaves are edible, young leaves are collected, washed and cooked before consumption. In Alduba village, Hamer-Benaworeda and South-Omo Farmers deliberately grow the species in their homes as food readily available.[9] Local healers use the plant for hypertension, catarrh and skin diseases.[10]

This study seeks to find the phytochemical component of *Leptadenia hastata* and its bioactivity as an alternative therapy for Fungal infection with less unwanted effects as compared to the harsh synthetic drugs which has unwanted effects that include; nephrotoxicity as a results of diuretic drugs, fever, chills, headache, nausea and vomiting. These problems necessitated the need for this research; to study the plant *Leptadenia hastata* (stem) which is being used by people and traditional herbalist as a vegetable and ethno medicine with little or no side effect as an adjuvant or remedy for burden of severe fungal infection. In this study we looked at the efficacy of the crude extract from this plant as an Ethno Medicine for *Aspergillus niger*, *Aspergillus flavus*, *Candida tropicalis* and *Fusarium oxysporium.*

MATERIAL AND METHOD

Sample collection

Leptadenia hastata Stem-bark were collected from the uncultivated farmland of Michika Local Government of Adamawa state. The plant *Leptadenia hastata* (yadiya) was dried under room temperature.

Preparation of samples

The stem-bark of the plant *Leptadenia hastata* was washed with distilled water to remove the soil and dust particles, they are thoroughly air dried and powdered using laboratory grinder machine (FGR-350, Quest Scientific). Extraction using hexane by placing 150g of the powdered samples into an Erlenmeyer flask and hexane 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 was then filtered using Whatman filter paper No.4 the residue was re-extracted with fresh hexane for another 72 h and filtered. Both extracts were combined and concentrated with a rotary evaporator (Heidolph Laborota 4000 efficient) under reduced pressure to obtain the hexane crude extract. The residues were re-extracted using a similar procedure with dichloromethane (CH2CLl2), followed by ethyl acetate (C2H5COOH), chloroform (CHCL3), and methanol (MeOH) to obtain dichloromethane, ethyl acetate, chloroform and methanol crude extracts, respectively. The dry weight and yield of each crude extracts were determined. It was then stored under a frozen condition until required.[11]

Antifungal Potential

The antifungal potential role 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 five days 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 with 100% inhibition.[12,13,14, and 11] 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 (1×108 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 preparation as reported by[11], 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 millilitres of sterile normal saline containing 3% w/v Tween 80 was used to disperse the spores with the aid of sterilized glass beads14. Standardization of the spore suspension to 1.0 x 106 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 and the zone of inhibition was measured every after 24hrs for five days.

RESULTS

The concentration of extract at days four and five, for 25ppm, 50ppm and 100ppm showed the highest activities against Aspergillus flavus, Aspergillus niger, Candida tropicallis, and Fusarium oxysporium. Thus, was very significant when compared with the control drug.

Aspergillus Niger

In table hexane stem extract of *Leptadenia hastata* inhibits the growth of Aspergillus niger, Aspergillus flavus, Candida tropicalis and Fusarium oxysporium in culture medium. higher in all the solvent extract concentration except for 250ppm, 500ppm and 1000ppm and at days 1-2 when compared with the control. Results showed that growth was inhibited in the presence of various concentrations of the stem-bark extract mixed in SDA culture at both days of observations. At 25ppm the growth of Aspergillus niger day five was 3.08±0.04mm close to control while100ppm was 3.32±0.08mm, higher than the control followed by 50ppm with 3.27±0.05mm. this result when compared to the different treatment days at the same concentration it shows a remarkable inhibition rate. The inhibition lowers as it increases in concentration from 250ppm -1000ppm on each fungus on the fifth day.

Aspergillus flavin

Similarly, the growth of Aspergillus flavin was also inhibited but the inhibition growth was also found to be more at 25ppm-250ppm concentrations. The inhibition was almost closer to that of the control 3.55±0.05mm, at concentration 100ppm, followed by 3.47±0.05mm, 3.22±0.04, and 3.00±0.09 respectively for 100ppm, 50ppm, 25ppm and slightly lower at 250ppm when compared with the control as well as to the different treatment days at the same concentration. However, the inhibition zone slightly drops at 500ppm and 1000ppm.

Candida tropicalis

Result shows that the growth of Candida tropicalis was inhibited by the hexane stem-bark extract at concentration of 50ppm higher when compared with inhibition effect of control on the fungus, which followed by 100ppm at day three and 25ppm at day five. This results of Candida tropicalis when compared with previous results of the effects of *Leptadenia hastata* stem bark on Aspergillus niger and flavin only extracts at concentration of 50ppm shows a good sensitivity. The growth of these fungi was affected enormously at 50ppm with 1.48±0.04 higher than the control 1.35±0.05. The inhibition of Candida tropicalis by this extract at 50ppm indicated dosage consciousness in the activities of the extract concentration on the fungus.

Fusarium oxysporium

Result shows that the growth of this fungus Fusarium oxysporium was also inhibited by hexane extract of *Leptadenia hastata* by only three concentrations in ascending order of 25ppm ,50ppm and 100ppm. The growth of these fungus was affected more at 100ppm. Result also shows that with the increase in plant extract concentration the rate of inhibition increases. Thus, in 25pp the inhibition was 1.38±0.04mm, at 50ppm inhibition was by 1.55±0.05mm, while 100ppm was by 1.68±0.04mm and less effective at 250ppm, 500ppm and 1000ppm respectively. The inhibition of *Fusarium oxysporium* by this extract is less compare to Aspergillus niger and Aspergillus Flavin. Generally, it shows that Aspergillus niger and Aspergillus flavin shows more sensitivity to hexane stem-bark extract when compared to the other fungi Candida tropicalis and Fusarium oxysporium.

Table 9: Effect of Hexane Stem-Bark Extract of *Leptadenia hastata* on Fungi.

			Concentration (ppm)					
Organisms	Day	Control	25	50	100	250	500	1000
Aspergillus niger	1	0.50 ± 0.00	0.42 ± 0.04	0.38 ± 0.04	0.30 ± 0.06	0.32 ± 0.04	0.23 ± 0.05	0.20 ± 0.05
	2	0.60 ± 0.09	0.60 ± 0.06	0.68 ± 0.04	0.77 ± 0.05[b]	0.53 ± 0.05	0.43 ± 0.05	0.28 ± 0.04
	3	1.55 ± 0.05	1.53 ± 0.05	1.47 ± 0.12	1.48 ± 0.04	1.43 ± 0.08	0.95 ± 0.05	0.40 ± 0.00
	4	2.48 ± 0.10	2.60 ± 0.06	2.68 ± 0.04	2.73 ± 0.08[b]	2.02 ± 0.04	1.53 ± 0.05	0.93 ± 0.05
	5	3.13 ± 0.08	3.08 ± 0.04[a]	3.27 ± 0.05[a]	3.32 ± 0.08[ab]	2.82 ± 0.08[a]	1.98 ± 0.04[a]	1.12 ± 0.12[a]
Aspergillus flavin	1	0.65 ± 0.05	0.53 ± 0.08	0.60 ± 0.00[d]	0.52 ± 0.08	0.48 ± 0.08[d]	0.33 ± 0.05[d]	0.28 ± 0.04[d]
	2	0.82 ± 0.04	0.70 ± 0.06	0.85 ± 0.05	0.95 ± 0.05[b]	1.00 ± 0.00	0.63 ± 0.05	0.38 ± 0.04
	3	1.23 ± 0.40	1.63 ± 0.05[d]	1.73 ± 0.08[bd]	1.57 ± 0.08[d]	1.50 ± 0.00[d]	1.37 ± 0.05[d]	0.55 ± 0.05
	4	3.22 ± 0.15	2.97 ± 0.05[d]	3.12 ± 0.08[d]	3.35 ± 0.05[bd]	2.85 ± 0.08[d]	1.95 ± 0.05[d]	1.02 ± 0.04[d]
	5	3.72 ± 0.10	3.22 ± 0.04[ad]	3.47 ± 0.05[ad]	3.55 ± 0.05[ad]	3.00 ± 0.09[ad]	2.60 ± 0.15[ad]	1.93 ± 0.08[ad]
Candida tropicalis	1	0.57 ± 0.08	0.40 ± 0.00	0.45 ± 0.00	0.67 ± 0.05[bd]	0.35 ± 0.05	0.28 ± 0.04	0.20 ± 0.04
	2	0.85 ± 0.05	0.70 ± 0.09	0.77 ± 0.05	0.97 ± 0.08	1.15 ± 0.05[abd]	0.82 ± 0.04[d]	0.53 ± 0.05[d]
	3	1.02 ± 0.09	0.78 ± 0.04	0.90 ± 0.00	1.15 ± 0.14	0.97 ± 0.08	1.10 ± 0.09[ab]	0.95 ± 0.05[ad]
	4	1.27 ± 0.10	1.07 ± 0.08	1.23 ± 0.05	1.30 ± 0.00[ab]	1.00 ± 0.09	0.93 ± 0.08	0.90 ± 0.11
	5	1.35 ± 0.05	1.25 ± 0.05[a]	1.48 ± 0.04[ab]	1.23 ± 0.08	1.05 ± 0.14	1.00 ± 0.00	0.90 ± 0.00
Fusarium oxysporium	1	0.63 ± 0.04	0.55 ± 0.05[d]	0.50 ± 0.06	0.50 ± 0.11	0.32 ± 0.04	0.30 ± 0.06	0.22 ± 0.04
	2	1.05 ± 0.05	0.95 ± 0.05[d]	1.00 ± 0.00[d]	1.25 ± 0.05[bd]	0.77 ± 0.05	0.47 ± 0.05	0.30 ± 0.00
	3	1.03 ± 0.10	1.15 ± 0.05	1.32 ± 0.04[b]	1.18 ± 0.10	1.00 ± 0.00	0.88 ± 0.04	0.77 ± 0.05
	4	1.52 ± 0.09	1.27 ± 0.05	1.38 ± 0.04	1.55 ± 0.05[b]	1.37 ± 0.08[a]	1.10 ± 0.06[a]	1.00 ± 0.00[a]
	5	1.53 ± 0.08	1.38 ± 0.04[a]	1.55 ± 0.05[a]	1.68 ± 0.04[ab]	1.25 ± 0.05	0.93 ± 0.05	0.72 ± 0.04

Values are Mean ± SD for six determinations

[a]Significantly ($p < 0.05$) higher compared to different treatment days at the same concentration on each fungus

[b]Significantly ($p < 0.05$) higher compared to the control on each fungus in each row

[d]Significantly ($p < 0.05$) higher compared to other fungi on the same day on each concentration

DISCUSSION

These studies are being carried out to study the fungal infection and the role of *Leptadenia hastata (per) decne* stem-bark to courtly the endemic danger of fungus in our community and the world at large. The antifungal properties of *Leptadenia hastata* stem-bark extract of different solvent (Hexane, Dichloromethane, Ehtylacetate, Chloroform and Methanol) against the pathogenic fungi viz. Aspergillus niger, Aspergillus flavus, Candida tropicallis and Fusarium oxysporium was studied for five days. The extract assayed by agar well diffusion method, the result inhibition rate of these extract was measured in diameter differences in the inhibition.

The difference in the inhibition rate of these selected microorganisms was because of the polarity difference of the extraction solvents used which sequentially decreases in the order of Methanol chloroform, Ethyl acetate, Dichloromethane and Hexane. Generally, it appears that the inhibitory effect of the plant extracts varies depending on the specific plant parts and solvent used as well as the fungal isolate with no specific trend related to the polarity of the solvent. When considering the different solvent systems used, a diversity of molecules with distinct polarities are extracted from plants. For example, Methanol and chloroform tends to extract a diversity of compounds such as polyphenols, glycosides and to some extent flavonoids that can be assumed to contribute to the inhibitory effect of the extract. Dichloromethane and hexane tend to extract mainly nonpolar constituents such as fats, fatty acid and terpenoids. Both the polar and nonpolar[15], constituents contributed to the antifungal activity of the plant extracts, although some fungal strains, especially Fusarium oxysporium were less susceptible to the hexane extracts of *Leptadenia hastata.* The sensitivity of a specific fungal isolate to a specific concentration is also worth considering. This would imply that the diverse fungal genetics and the selectivity of specific plant part constituents are important when developing either animal or plant fungicides as well as the inhibition duration also has an important role in optimum result as well as the growth phase of microorganism that fluctuate with increase in days which is in proportion with increases in inhibition zone. The highest activity in this study was observed at days five of incubation period and many researchers also reported that the optimal result was obtained from 4-5 days of incubation for most of the fungi but depending on extract types and concentration.[16,17]

Thus, care should therefore be exercised as this could result in the selection of a specific resistant genotype that can either be less or be more mycotoxigenic. For example, the hexane, dichloromethane, ethyl acetate, chloroform and methanol stem-bark extract of *Leptadenia hastata.*

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Author contributions

Isaac John Umaru and Fasihuddin Ahmad Badruddin conceived the research project. Hauwa A. Umaru and Zaini B Assim contributed to the writing, and provided critical inputs to the contents of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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