

Antifungal Activity of *Leptadenia hastata* (Pers) Decne Leaves Extract

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Received: 13.11.2017 | Revised: 20.12.2017 | Accepted: 26.12.2017

ABSTRACT

Objective: The aim of this study was to investigate the antifungal activities of the hexane extract of *Leptadenia hastata*. The Concern has been expressed about the rising prevalence of pathogenic microorganisms which are resistant to the modern antimicrobial drugs as such the search for drugs and dietary supplements derived from plants have accelerated in recent years. Ethno pharmacologists, biochemist, botanists, microbiologists and natural product scientists are combing the earth for phytochemicals and leads which could be developed for treatment of infectious diseases. **Materials 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 day four and five, 25ppm, 50ppm and 100ppm showed the highest activities against *Aspergillus flavus* and *Aspergillus niger*. While the inhibition of *Candida tropicalis*, and *Fusarium oxysporium* was very slow when compared with the other two at the same concentration same days. The activity of the extracts was determined against different pathogenic fungi including. *Aspergillus flavus*, *Aspergillus niger*, *Candida tropicalis*, and *Fusarium oxysporium*. Extracts at 50ppm and 100ppm were the most effective followed by 500ppm which showed moderate activities. The lowest activity was recorded for 1000ppm. The Four fungi differed with regard to their susceptibility to the plant concentration per days. *Aspergillus niger*, was the most susceptible, followed by *Aspergillus Flavus*, *Candida tropicalis* and *Fusarium oxysporium*, respectively. **Conclusion:** the study indicate that hexane extracts at a given concentration possess an antifungal potential which is effective at 25ppm, 50ppm and 100ppm, hence should be used to treat infections with pathogenic fungi.

Key words: Antifungal, Diflucan, *Leptadenia hastata*, Dimethyl sulfoxide

INTRODUCTION

Concern has been expressed about the rising prevalence of pathogenic microorganisms

which are resistant to the modern antimicrobial drugs that have been produced in the last three decades^{4,5}.

Cite this article: Umaru, I. J., Badruddin, F.A., Assim, Z.B. and Umaru, H.A., Antifungal Activity of *Leptadenia hastata* (Pers) Decne Leaves Extract, *Int. J. Pure App. Biosci.* 5(6): 14-18 (2017). doi: <http://dx.doi.org/10.18782/2320-7051.5996>

This has been a treat to health as a result of the high cost, adulteration and increasing toxic side effects of these synthetic drugs coupled with their inadequacy in diseases treatment found more especially in the developing countries cannot be over emphasized²⁰. Among the pathogenic microorganism fungal diseases is one of the most threatening and major cause of morbidity and mortality worldwide.² As a result of the number of multi-drug resistant microbial strains and the appearance of strains with reduced susceptibility to antibiotics continuously increasing. This increase has been attributed to indiscriminate use of broad spectrum antibiotics and immunosuppressive agents^{3, 4}. Coincidentally, the last decade has also witnessed increasing intensive studies on extracts and biologically active compounds isolated from plant species used for natural therapies or herbal medicine.^{5, 7}

Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs⁸. A wide range of medicinal plant parts is used for extract as raw drugs and they possess varied medicinal properties. While some of these raw drugs are collected in small quantities by the local communities and traditional healers for local use¹⁹. Considering the vast potentiality of plant as sources for antimicrobial drugs with reference to antibacterial and antifungal agents, an investigation as regard the plant *Leptadenia hastata* was undertaken to screen the local flora for its antifungal activity. This situation provided the impetus to the search for new antifungal substances from various sources like *Leptadenia hastata*. Traditional medicine has made use of many different plant extracts for treatment of fungal infections and some of these have been tested for in vitro antifungal activity⁶.

Leptadenia hastata (Pers) Decne (Family-Asclepiadaceae), commonly known as hastata is edible non-domesticated vegetable and it is collected in wild throughout Africa⁹.

Leptadenia hastata is a voluble herb with creeping latex stems, glabrescent leaves, glomerulus and racemus flowers as well as follicle fruits. It is typically grown in tropical dry lands in sandy soil¹⁶. Wild foods like *Leptadenia hastata* provide food security during seasonal changes and are used medicinally in many areas. The breeders commonly used the leaf and stems for their parasitic activity and against placental retention.^{17, 1, 18}.

Vernacular names for *L. hastata* include: *hagalhadjjar* (Arabic) in Chad, *yadiya* (Hausa) in Nigeria and Niger, *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.^{9, 11}. The present study was designed to evaluate the in vitro anti-fungal activity of *Leptadenia hastata* hexane leave extract against four strain of fungi, *Aspergillus niger*, *Aspergillus flavin*, *Candida tropicalis* and *Fusarium oxysporium*.

MATERIAL AND METHOD

Sterilization of materials used

All glass wares were thoroughly washed in water containing detergent and rinsed with distilled water, they were air dried and sterilized in the oven at 160°C for one hour. Inoculating chamber and growth chamber were fumigated using formaldehyde and then irradiated on exposure to UV lamp for one hour. Laboratory benches were cleaned with absolute alcohol while the inoculating loop was flamed to redness and allowed to cool prior to use.

Leptadenia hastata leaves

Freshly harvested leaves of *Leptadenia hastata* was used for the preparation of the crude extract. It was collected from an uncultivated farm land in Michika LGA of Adamawa State-Nigeria. It was authenticated Ahmadu Bello University Zaria and Voucher No PU: 2 ABU Herbarium No 900220, the plant was dried under room temperature.

Drug preparation

The freshly dried leaves of *Leptadenia hastata* was grounded into fine powdered form using laboratory mortar and pestle and electric blender. 150mg of the powdered leaf was weighed into a beaker and mixed with distilled water three times the quantity of the sample and allow to stand for two days with continues shacking at time interval for 12hrs. The mixture was then filtered using Whatman filter paper No.4 and the solvent was then evaporated using a rotary evaporator (Heldolph Laborato 400). It was then stored under frozen condition for further use.

Reconstitution of the extracts

2 g of the extract was weighed and dissolved in 20ml of 50% Dimethyl sulfoxide (DMSO) to make a stock concentration of 100mg/ml from which the various concentrations used were calculated.

Microorganisms used

Isolates of *Aspergillus flavin*, *Aspergillus niger*, *Candida tropicalis*, and *Fasarium oxysporium* from four different Concentration were used. They were collected from Nigeria Institute of Medical research Yaba (NIMIR), where they were maintained on slants.

Drug used

Fluconazole common name Diflucan (Pfizer Inc New York, NY) was used as reference standard for antifungal studies.

Antifungal Activity

The antifungal activities of the extracts were tested using various pathogenic fungi. The antifungal activities of the extracts were performed by agar disc diffusion method^{12, 13, 15}. Dimethyl sulfoxide DMSO was used as a negative control and Fluconazole common name Diflucan was used as a positive control. The plates were done in triplicates and were incubated at 37 °C. The antimicrobial activity was taken on the basis of diameter of zone of inhibition, 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). 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 24hrs 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 beads¹⁴. 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 24h¹⁰.

RESULTS AND DISCUSSION

Results

Result obtained from the studies is presented in table 1. Results obtained reveled that all the fungal species were affected by the administration of *Leptadenia hastata* in a dose dependent manner. The inhibition of the microorganism significantly increases at ($P < 0.05$) at 50ppm 100ppm and 500ppm when compared with control.

Table 1: Effect of Hexane Leaf Extract of *Leptadenia hastata* on Fungi

| Organisms | Day | Concentration (ppm) | | | | | | |
|----------------------------|-----|---------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| | | Control | 25 | 50 | 100 | 250 | 500 | 1000 |
| <i>Aspergillus niger</i> | 1 | 0.92 ± 0.04 | 0.90 ± 0.06 ^d | 0.97 ± 0.10 ^b | 0.65 ± 0.16 | 0.82 ± 0.12 ^d | 0.52 ± 0.12 | 0.45 ± 0.19 |
| | 2 | 1.03 ± 0.12 | 0.63 ± 0.12 | 0.95 ± 0.10 ^d | 0.58 ± 0.10 | 1.05 ± 0.08 | 0.90 ± 0.06 ^d | 0.60 ± 0.06 ^d |
| | 3 | 2.86 ± 0.11 | 2.38 ± 0.23 ^d | 2.42 ± 0.31 ^d | 2.12 ± 0.18 ^d | 1.82 ± 0.15 | 1.43 ± 0.14 | 0.92 ± 0.08 |
| | 4 | 3.71 ± 0.14 | 3.05 ± 0.10 ^d | 3.20 ± 0.14 ^d | 2.98 ± 0.17 ^d | 2.02 ± 0.50 | 2.40 ± 0.24 ^d | 1.47 ± 0.14 ^d |
| | 5 | 4.67 ± 0.12 | 3.48 ± 0.17 ^{ad} | 3.75 ± 0.55 ^{ad} | 3.12 ± 0.21 ^{ad} | 2.65 ± 0.20 ^{ad} | 3.32 ± 0.19 ^{ad} | 1.90 ± 0.06 ^{ad} |
| <i>Aspergillus flavin</i> | 1 | 0.87 ± 0.05 | 0.75 ± 0.10 | 0.85 ± 0.08 | 0.67 ± 0.10 | 0.72 ± 0.08 | 0.60 ± 0.09 | 0.55 ± 0.10 |
| | 2 | 0.83 ± 0.08 | 0.75 ± 0.08 | 0.85 ± 0.08 | 0.82 ± 0.08 | 1.08 ± 0.08 ^{bd} | 0.83 ± 0.05 | 0.60 ± 0.13 ^d |
| | 3 | 2.05 ± 0.05 | 1.82 ± 0.45 | 2.00 ± 0.13 | 1.95 ± 0.10 | 1.85 ± 0.10 ^d | 1.40 ± 0.19 | 0.95 ± 0.05 |
| | 4 | 3.08 ± 0.08 | 3.02 ± 0.13 | 2.73 ± 0.37 | 2.88 ± 0.08 | 2.45 ± 0.24 ^d | 1.60 ± 0.25 | 1.28 ± 0.15 |
| | 5 | 3.46 ± 0.05 | 3.25 ± 0.27 ^a | 3.00 ± 0.26 ^a | 3.02 ± 0.46 ^a | 2.48 ± 0.15 ^a | 3.17 ± 0.23 ^a | 1.53 ± 0.12 ^a |
| <i>Candida tropicalis</i> | 1 | 0.82 ± 0.08 | 0.83 ± 0.12 | 1.03 ± 0.08 ^{bd} | 0.87 ± 0.06 ^d | 0.72 ± 0.12 | 0.52 ± 0.13 | 0.53 ± 0.15 |
| | 2 | 1.00 ± 0.6 | 0.87 ± 0.05 ^d | 0.90 ± 0.06 | 0.85 ± 0.08 ^d | 1.00 ± 0.06 | 0.82 ± 0.07 | 0.60 ± 0.06 ^d |
| | 3 | 2.10 ± 0.09 | 1.95 ± 0.10 | 2.07 ± 0.14 | 1.98 ± 0.08 | 1.60 ± 0.09 | 1.53 ± 0.12 | 1.15 ± 0.16 ^d |
| | 4 | 2.39 ± 0.08 | 2.00 ± 0.09 | 2.15 ± 0.24 | 2.07 ± 0.33 | 1.90 ± 0.09 | 1.48 ± 0.21 | 1.05 ± 0.12 |
| | 5 | 3.10 ± 0.08 | 2.97 ± 0.05 ^a | 2.80 ± 0.14 ^a | 2.28 ± 0.16 ^a | 2.17 ± 0.24 ^a | 2.82 ± 0.26 ^a | 1.35 ± 0.10 ^a |
| <i>Fusarium oxysporium</i> | 1 | 0.82 ± 0.08 | 0.58 ± 0.08 | 0.77 ± 0.08 | 0.70 ± 0.09 | 0.68 ± 0.08 | 0.93 ± 0.10 ^{bd} | 0.88 ± 0.17 ^{bd} |
| | 2 | 0.85 ± 0.05 | 0.62 ± 0.10 | 0.63 ± 0.05 | 0.70 ± 0.06 | 0.98 ± 0.08 ^b | 0.80 ± 0.09 | 0.53 ± 0.12 |
| | 3 | 1.97 ± 0.08 | 1.98 ± 0.08 | 1.97 ± 0.08 | 2.00 ± 0.06 | 1.62 ± 0.08 | 1.57 ± 0.12 ^d | 1.07 ± 0.10 |
| | 4 | 2.09 ± 0.08 | 1.97 ± 0.05 | 2.08 ± 0.15 | 1.92 ± 0.08 | 1.77 ± 0.12 | 1.18 ± 0.23 | 0.93 ± 0.14 |
| | 5 | 2.26 ± 0.10 | 2.07 ± 0.08 ^a | 2.15 ± 0.19 ^a | 2.05 ± 0.10 ^a | 1.97 ± 0.16 ^a | 2.13 ± 0.20 ^a | 1.10 ± 0.17 ^a |

Values are Mean ± SD for six determinations

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

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

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

DISCUSSION

Table 1. Indicated the antifungal activity of the extracts of *Leptadenia hastata* from six different concentration of the hexane extracts. The result of the MIC studies revealed the anti-fungal activity of the extracts against the tested strain of the microorganism between concentration ranges of 25ppm, 50ppm, 100ppm, 250ppm 500ppm to 1000pp. The result of zone of inhibition study revealed that the extracts possess anti-fungal activity in concentration dependent manner against the test organism and were comparable with the standard drug

Mean ±SD yield of various extracts concentration confirm that 100ppm of the extracts has minimum yield with 0.85± 0.05 at day one and maximum yield at 3.17±0.05 day five for *Aspergillus niger*, were as *Aspergillus flavin* has maximum inhibition at 3.78±0.04. Hexane extract was found to be less active against *Candida tropicalis* and *Fusarium oxysporium* when compared with the other two at the same time and concentration as shown in table one. Though the extract exhibits its potency against *Candida tropicalis* and *Fusarium oxysporium*. The hexane extract imparts significant antifungal activity against all test strains whereas extract is more potent

against *Aspergillus niger* and *Aspergillus flavin*.

This suggests that the plant parts of *Leptadenia hastata* have a broad spectrum of activity, although the degree of susceptibility could differ between different organisms. The broad spectrum of antifungal activity found in this present study may be attributed to the presence of secondary metabolites of various chemical types present in the plant material. Our results indicates the potential usefulness of *Leptadenia hastata* in the treatment of various pathogenic diseases as it may help in the discovery of new chemical classes of antifungal as well as antibiotic that could serve as selective agents for the maintenance of human health and may provide biochemical tools for the study of fungal infectious diseases. The discovery of a potent remedy from plant origin will be a great advancement in fungal infection therapies.

CONCLUSION

The outcome of this study show that the plants investigated possess antifungal activities, thus justifying their use in folk medicine for the treatment of skin and other related infections.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

The authors are thankful to the Natural Product laboratory FRST/FSTS University Malaysia Sarawak for the enabling support to carry out this study.

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