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Antimycotic Effect of the Aqueous Leaf Extract of *Pterocarpus erinaceus* in Rats

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Abstract: The aqueous leaf extract of *Pterocarpus erinaceus* (Leguminoceae) was investigated for possible antimycotic effect in Sprague dawley rats. The extract was tested against moulds (*Aspergillus niger* and *Aspergillus flavus*) and dermatophytes (*Trichophyton rubrum* and *Microsporum gypseum*). The extract at 20-40 mg kg^{-1} body weight significantly ($p<0.05$) and dose dependently inhibited the growth rate of the moulds and dermatophytes by over 60 and 97%, respectively. In the *in vivo* study, there was also a significant reduction in the number of dermatophyte spores recovered from the infected sites treated with the extract as compared with the non treated sites. The extract produced no sign of acute toxicity or death when a limit dose of 2 g kg^{-1} body weight was administered orally in rats. Collectively, these results suggest that the extract possess antimycotic effect and appears to be safe when given orally at a limit dose of 2 g kg^{-1} body weight of the rats. This therefore supports the use of *Pterocarpus erinaceus* leaf extract traditionally for the treatment of fungal skin diseases.

Key words: Antimycotic, griseofulvin, *Pterocarpus erinaceus*, *Trichophyton rubrum*

INTRODUCTION

Pterocarpus erinaceus (Leguminoceae) commonly known as winged fruit tree is a shrub or small 3-6 m deciduous flowering savannah tree which is widely distributed in West and Central Africa. It is very popular among the Hausa speaking people of Northwestern Nigeria because of its medicinal properties. Decoctions made from various parts of the plant are used locally for the treatment of both human and animal diseases. Fresh leaf from the plant is crushed and applied externally to treat fungal skin diseases such as athlete foot, ring worm and eczema. The grated root is mixed with tobacco and smoked in a pipe as a cough remedy. The stem bark decoction is taken orally for gastrointestinal upsets (International Centre for Research in Agroforestry (ICRAF), 1998).

Majority of the people in third world countries live in rural areas where access to modern health care services is limited. Consequently, they rely heavily on herbal medicine for treatment of their illnesses. There is therefore a need to evaluate widely used medicinal plants such as *Pterocarpus erinaceus* for both efficacy and safety. Superficial mycotic infection is rampant in this area due to the poor level of personal and environmental hygiene, poverty, overcrowding and animal husbandry (Ameh and Okolo, 2004). Mycosis is one condition alleged to be successfully treated with *Pterocarpus erinaceus* leaf extract in these communities.

The antimalarial (Karou *et al*., 2003), anthelmintic (Maidou *et al*., 2005) and antigonadotropic (Benie *et al*., 2003) activities of *Pterocarpus erinaceus* extract have been investigated but its antimycotic activity to our knowledge, not yet reported. This study examined the antimycotic activity and the safety of the plant extract following animal exposure.

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MATERIALS AND METHODS

Collection and Extraction of the Plant Leaves

Fresh leaves of *Pterocarpus erinaceus* were collected in the month of September 2005 from its natural habitat at Kibiyari village in Sanyinna local government area of Sokoto State, Nigeria. The plant was authenticated by Auwal Umar of Biological Sciences Department, Usmanu Danfodiyo University (UDUS), Sokoto, Nigeria. A Voucher specimen (No. : A-DIPE-2) was deposited at the Herbarium, Department of Pharmacology, UDUS for future reference.

The leaves were air dried to constant weight and pulverized to a dry powder. Hundred gram of the powder was measured and thoroughly mixed with 2 L of distilled water. The mixture was allowed to stand for 24 h. It was then filtered and the filtrate was freeze dried at a temperature of -17°C. The percentage yield of extract was calculated to be 16.3% (w/w). A series of concentrations (5, 10, 20 and 40 mg mL^{-1}) of the extract were made in distilled water and used in the subsequent experiments.

Experimental Animals

Adult Sprague dawley rat of either sex, weighing 150-200 g, were used for this study. The animals were bred and maintained in the experimental animal house of the Pharmacology Department, College of Health Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria. They were fed with standard rat feed (Vital Feed, Jos, Nigeria) and given free access to tap water under a well ventilated condition of 12 h light, 12 h dark cycle. The study was conducted in accordance with the Organization for Economic Cooperation and Development (OECD, 2001) principles on good Laboratory practice.

Test Organisms

The microorganisms used for the screening include: *Aspergillus niger*, *Aspergillus flavus*, *Trichophyton rubrum* and *Microsporum gypseum*. The moulds (*A. niger* and *A. flavus)* with identification number [AS:2-326(BSDS)] were standard and packaged organisms obtained from the Mycology unit of the Biological Sciences Department, Usmanu Danfodiyo University, Sokoto, Nigeria. The dermatophytes namely *Microsporum gypseum* and *Trichophyton rubrum* were grown from clinical isolates. The isolates were obtained with consent from infected pupils of a local Islamic school in Sokoto, Nigeria.

The pupils were randomly selected and physically screened for ringworm lesions on the scalp (tinea capitis). The infected portion of the scalp was first sterilized with methylated spirit and then scraped unto a sterile filter paper with the aid of a sterile surgical blade. The organisms were cultured in Sabouraund Dextrose Agar (SDA) medium incorporated with 500 mg of chloramphenicol to inhibit the growth of any bacterial contaminant. Subcultures were made to obtain isolates which were identified by microscopy as pure colonies of *M. gypseum* and *T. rubrum*, with the aid of their spores and hyphae (Cheesbrough, 1982).

Drugs and Chemicals

The drugs and chemicals used in this study include: Griseofulvin tablets (Grisovid(R)), obtained from HOVID BHD, Malaysia (Batch No. AFO 8513); Potato Dextrose Agar (PDA) prepared according to the manufacturer's specification (Oxoid Ltd. Basing stoke, Hants, England); Malt Extract Agar (MEA) also prepared according to the manufacturer's guide (GMbH and Co. D - 3440, Eschwege Germany) and Sabouraund Dextrose Agar (SDA).

Acute Oral Toxicity (Limit Test)

A limit test dose of 2 g kg^{-1} body weight as described by Organization for Economic Coorperation and Development Guideline 425 (2000) and Interagency Research Animal Committee (IRAC, 2004) was used in this study. Five healthy adult rats of either sex were used. The female were non-pregnant and their ages were between 8 and 12 weeks. Each animal was dosed in sequence at

interval of 48 h with 2 g kg^{-1} body weight of the extract in 2 mL of distilled water. The animals were observed individually for any sign of acute toxicity, morbidity or mortality during the first 24 h and thereafter daily, for a total of 14 days.

Antimycotic Activity Evaluation

In vitro Assessment of Antimycotic Activity

Agar dilution method (Taudou, 1990) was used to assess the antimycotic activity of the leaf extract *in vitro*. Potato Dextrose Agar (PDA) and Malt Extract Agar (MEA) were used as culture media for the moulds (*A. niger* and *A. flavus)* and the dermatophytes (*Trichophyton rubrum* and *Microsporum gypseum*), respectively. Two hundred milliliter each of 5, 10, 20 and 40 mg mL^{-1} concentrations of *Pterocarpus erinaceus* leaf extract in PDA and MEA were prepared by autoclaving at 121°C for 15 min. Fifty milliliter aliquot of each was poured in sterile Petri-dishes before it cooled down. Plain, unincorporated PDA and MEA were also poured to serve as negative control and another set incorporated with 40 mg mL^{-1} Griseofulvin (a standard antimycotic agent) poured, to serve as positive control.

Two millimeter discs of the test organisms, punched with cork borer from the edge of actively growing culture plates (7-days-old for the mould and 10-days -old for the dermatophytes - slow growers) were inoculated in the centre of the incorporated media plates and the controls, with the aid of a sterile inoculating needle. The plates were inoculated in triplicates and labelled according to the concentrations of the extract and their test organisms in the respective culture medium. They were then incubated at 35±2°C for seven days. The diameter of growth of each of the test organism in each plate was measured daily by linear method along three planes. The mean of three measurements were recorded as the daily reading for each plate. The percentage inhibition was thereafter calculated using the following formula:

$$1\% = \frac{dc - dt}{dc} \times 100$$

Where:

dc = Diameter of control culture
dt = Diameter of treated colony
I% = Percentage inhibition

In vivo Assessment of Antimycotic Activity

The method of Niwano *et al.* (1998) was slightly modified and adopted for the assessment of the antimycotic effect of *Pterocarpus erinaceus* aqueous leaf extract on the rat skin infected with *Trichophyton rubrum* and *Microsporum gypseum*. A total of sixty Sprague Dawley rats of either sex weighing 150-200 g were used for this study. Initially thirty rats were randomly selected and divided into six groups of five rats each. The hairs on the dorsal surface of the body of the rats were carefully shaved, without inflicting any wound on the skin. The shaved area was gently smeared with 0.2 mL of the spores (3.6×10^2 mL^{-1}) *Microsporum gypseum*. The application was repeated once daily for five days and thereafter, the animals were observed daily until there was growth of the organisms.

The growth of *M. gypseum* manifested on the skin surface of the rats between the twelfth and the thirteenth day. Treatment was then commenced immediately with the various concentrations of the leaf extract and griseofulvin. The rats in group 1 were treated orally with 40 mg kg^{-1} body weight of griseofulvin while those in groups 2, 3, 4 and 5 received 5, 10, 20 and 40 mg kg^{-1} of the extract, respectively through the oral route daily for 14 days. The animals in group 6 were left untreated to serve as control. After the 14th day of treatment, the numbers of Microsporum spores available on the site of the infection were determined by collecting the washings from the infected area and counting the spores under the microscope at x10 objective.

The same procedure was repeated using the other thirty rats which were infected with *Trichophyton rubrum* and treated with various concentrations of the extract and griseofulvin.

Statistical Analysis

All the data were expressed as the mean±standard error of the mean (SEM). One way analysis of variance (ANOVA) with subsequent Dunnet's post hoc analysis was used to assess further differences between groups. Values of $p<0.05$ were considered significant. The analyses were performed with Instat statistical package (Graph Pad Software. Inc. USA).

RESULTS

Acute Toxicity

The limit dose of 2 g kg^{-1} body weight did not produce any mortality or sign of acute toxicity in the animals during observation.

In vitro Antimycotic Activity

The *in vitro* assay of antimycotic activity is shown in Table 1. The aqueous extract of *Pterocarpus erinaceus* at a concentration of 40 mg mL^{-1} produced a complete inhibition (>97%) of the *Trichophyton rubrum* and *Microsporum gypseum*. This was higher than that of griseofulvin (92%) a standard antimycotic agent. The inhibitory effect of the extract on the mould (*A. flavus* and *A. niger*) was less (>60%) compared with that of the dermatophyte. The extract produced an insignificant inhibitory activity (<50%) at concentrations of 5 and 10 mg mL^{-1}.

In vivo Antimycotic Activity

Pterocarpus erinaceus aqueous leaf extract produced a significant ($p<0.05$) and dose dependent reduction in the number of *Trichophyton* and *Microsporum* spores recovered from the site of the infection after 14 days treatment, as shown in Table 2. The effect of this extract at a concentration of 40 mg mL^{-1} was similar to that of Griseofulvin at the same concentration. There were obvious differences observed in the physical appearance (disappearance of rashes and appearance of pinkish skin colour) of the infected skin areas of the animals before and after treatment with the extract.

Table 1: Inhibition of selected moulds and dermatophytes with aqueous leaf extract of *Pterocarpus erinaceus* cultured *in vitro*

	Inhibition (%)			
Concentrations of extract (mg mL^{-1})	*A. niger*	*A. flavus*	*Trichophyton*	*Microsporum*
5	38.3	29.7	41.1	42.1
10	53.2	47.3	62.6	64.3
20	62.0	48.1	69.8	76.1
40	63.4	60.8	97.3	98.2
GS	65.3	69.2	92.3	86.6

GS = Griseofulvin; 50-70% indicates strong inhibitions; 71-100% very strong inhibitions

Table 2: Reduction of *Trichophyton and Microsporum* spores after treatment with various concentrations of *P. erinaceus* leaf extract

	No. of spores (S mL^{-1})	
Concentrations of extract (mg mL^{-1})	*Microsporum*	*Trichophyton*
5	39.3±0.3	36.7±0.1
10	25.0±0.6	20.7±1.2
20	18.1±0.9	13.0±0.6
40	0.0±0.0	0.0±0.0
GS	0.0±0.0	0.0±0.0
NC	86.0±0.2	73.0±0.0

S: Spores; Gs: Griseofulvin; NC: Negative Control (Non treated); values expressed as Mean±SEM on 5 observations. $p<0.05$ (NC vs. treatment)

DISCUSSION

This study shows that the LD_{50} of the extract is greater than 2 g kg^{-1} body weight of the rats when administered orally. The European chemical Industry ecology and toxicology guideline (International Research Animal Committee (IRAC), 2004) considers LD_{50} above 2 g kg^{-1} as likely to be non-toxic.

The aqueous leaf extract of *Pterocarpus erinaceus* at a concentration of 40 mg mL^{-1} produced a complete inhibition of the dermatophyte and more than 60% inhibition of the moulds (Table 1). *Aspergillus* species (moulds) cause mainly systemic mycotic diseases while *Trichophyton rubrum and Microsporum gypseum* (dermatophytes) produce superficial infections (Aguiyi, 2006). It has been observed that antimycotic agents act selectively on either the systemic or superficial infections (Schiwarz and Kaulfman, 1977) and this probably is responsible for the difference in percentage susceptibility of the moulds and dermatophytes seen in this study. The effect of this extract at a concentration of 40 mg mL^{-1} was similar to that of Griseofulvin at the same concentration. Griseofulvin is a conventional antimycotic agent, which act by interfering with microtubule function of nucleic acid synthesis and polymerization. Its clinical use is mostly against superficial mycosis (Carwight, 1978).

The finding that oral administration of the extract for 14 days eliminates the spores of *Trichophyton* and *Microsporum* from the site of infection in the rats is equally significant (Table 2). A plant extract can possess *in vitro* but without any *in vivo* activity. Agaie *et al*. (2005) reported that, the leaf extract of another medicinal plant *Anogeissus leiocarpus* demonstrated *in vitro* anthelmintic effect but limited *in vivo* activity. *Pterocarpus erinaceus* has shown both *in vitro* and *in vivo* activity against *Microsporum* and *Trichphyton* species comparable to griseofulvin.

CONCLUSION

This study has shown that the aqueous leaf extract of *Pterocarpus erinaceus* is safe for use. It possesses antimycotic activity against *Aspergillus niger*, *Aspergillus flavus*, *Trichophyton rubrum* and *Microsporum* gypseum. The effect against dermatophytes was stronger than that of the moulds. These findings are therefore in support of the traditional use of *Pterocarpus erinaceus* leaf extract in the treatment of fungal skin diseases such as ringworm, eczema and athlete foot.

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