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Preliminary Phytochemical and Antifungal Screening of the Aqeous Extract of the Leaves of *Pterocarpus erinaceous*

[1]K. Abubakar, [2]M.E. Halilu, [3, 5]A.O. Jimoh and [4]A.S. Hudu
[1]Department of Pharmacology, [2]Department of Pharmacognosy and Ethnopharmacy,
Faculty of Pharmaceutical Sciences,
Usmanu Danfodiyo University, Sokoto, Nigeria
[3]Department of Pharmacology, [4]Department of Pathology and Microbiology,
College of Health Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria
[5]Department of Health Services, Usmanu Danfodiyo University, Sokoto, Nigeria

Abstract: Qualitative phytochemical analysis of the aqeous extract of *Pterocarpus erinaceous* indicated that it contains tannins, alkaloids, saponins, flavonoids, volatile oils and glycosides. Antifungal testing of the aqueos extract on moulds and dermatophytes shows antimycotic activity comparable to Griseofulvin at concentrations of ≥ 40 mg mL^{-1}.

Key words: *Pterocarpus erinaceous*, phytochemistry, antifungal screening, dry forests, speaking tribes, Nigeria

INTRODUCTION

Pterocarpus erinaceous (leguminoceae) is a decidous legume tree of African savannahs and dry forests. It is called madobiya among the Hausa speaking tribes of Northern Nigeria. It is also called palissondre in Senegal. Among the Hausas, it is used for the treatment of fungal infections such as ringworm, eczema and athletes foot. The leaves are used as a febrifuge, the bark is used for tooth and mouth troubles and as astringent for severe diarrhoea and dysentery (ICRAF, 1998).

There is a wide spread use of the plant among traditional healers for a variety of human and animal diseases. It also serve as an excellent animal feed because of its high protein content (ICRAF, 1998). In developing countries like Nigeria, majority of people rely heavily on herbal medicines for their treatment due to high cost of the orthodox drugs, incidence of drug resistance and side effect (Arora and Kaur, 1999).

Since, developing local medicines may be cheaper than exporting synthetic drugs, it is of particular importance to undertake research into the toxicity profile of herbal medicines and to isolate the active ingredient(s) that is responsible for the claimed pharmacological activity.

Many studies indicate that in higher plants there are many phytochemicals which confer in most instances the pharmacological properties of the plants. The antifungal, antibacterial or antiviral properties is mostly dependent on these plant phytochemicals (Holetz *et al*., 2002; Horvath *et al*., 2002; Jantan *et al*., 2003; Khan *et al*., 2003; Perez, 2003).

MATERIALS AND METHODS

Collection of plant materials and extract preparation: The plant materials from *Pterocarpus erinaceous* were collected in the month of September, 2005 from its natural habitat at Kibiyari village, Sanyinna local government area, Sokoto state, Nigeria. The plant was identified by a taxonomist in the Department of Biological Sciences, Usmanu Danfodiyo University Sokoto, Nigeria. A voucher specimen (No.: ADIPE-2) was deposited at the Department of Pharmacology, UDUS for reference.

Drugs and test organism: The standard drug used for this experiment, Griseofulvin Grisovid^{R} was obtained from Hovid BHD Malaysia (Batch no. AFO, 8513), Potato Dextrose Agar (PDA) prepared according to manufacturers specification (Oxoids Ltd., Basing stoke, Hants, England); Malt Extract Agar (MEA) also prepared according to the manufacturers guide (GMbH and Co. D-3440, Eschwege Germany) and Sabouraud Dextrose Agar (SDA).

Corresponding Author: K. Abubakar, Department of Pharmacology, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria

Phytochemical analysis: The phytochemical analysis was carried out according to standard procedures (Trease and Evans, 1978; Harbone, 1973).

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 Mycology unit of Department of Biological Sciences, 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 1st sterilized with methylated spirit and then scraped unto a sterile filter study with the aid of a sterile surgical blade. The organisms were cultured in Malt Extract Agar (MEA) medium incorporated with 500 mg of chloramphenicol to inhibit the growth of any bacteria contaminant. Sub-cultures 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).

Standardization of the innoculum: The solidified Potato Dextrose Agar (PDA) was inoculated with stock mycelia of *Aspergillus niger* and *Aspergillus flavus*. *Trichophyton* and *Microsporum* sp. were also inoculated into the malt extract agar. The organisms were allowed to grow at room temperature (27-30°C for 7 days. Thereafter, sterile cork borer (2×2 mm) was used to punch the plates in other to obtain a uniform innocolum size.

Antifungal activity determination: Antifungal screening was performed using Agar incoporation method as described by Taudou. About 200 mL of 5, 10, 20 and 40 mg mL^{-1} concentrations of *Pterocarpus erinaceous* leaf extract in PDA and MEA were prepared by autoclaving at 121°C for 15 min. About 50 mL of the extract were added to the flasks containing the PDA/MEA they were again swirled to mix thoroughly before they were aseptically poured into sterile petri-dishes.

The dishes were appropriately labeled according to their concentrations and their test organisms, respectively. About 2 mL disc of the test organisms punched with cork borer from the edge of actively growing culture plates were inoculated in the center of the incorporated media plates and the controls with the aid of a sterile inoculating needle. The plates were inoculated in triplicates and labeled according to the concentrations of the extract and their test organism in the respective culture medium. They were then inoculated at 35°C for 7 days. The diameter of growth was measured daily along two planes by the Linear method (Lilly, 1951). The mean of the measurements were recorded daily as the daily reading for each plate.

RESULTS AND DISCUSSION

The results of the preliminary phytochemical analysis revealed the presence of alkaloids, tannins, flavonoids, volatile oils glycosides, saponins and anthraquinones (Table 1). The result of the phytochemical analysis revealed the presence of saponins, glycosides, volatile oils, flavonoids, alkaloids tannins and reducing sugars. The pharmacologic properties of *Pterocarpus erinaceous* may be linked to the presence of these secondary metabolites.

Moreover, the research of Leven *et al.* (1979) and Abdulrahman (1992) provides a link between plant phytochemicals and antimicrobial properties. It has also been reported that the presence of compounds such as alkaloids, tannins, saponins and phenols in medicinal plants confer antifungal activity (Gundidza *et al*., 1992; Renault *et al*., 2003). The result of the *in vitro* studies as shown in Table 2 showed that the extract at a

Table 1: Preliminary phytochemical screening of the aqueous leaf extract of *Pterocarpus erinaceous*

Tests	Result
Alkaloids	+
Tannins	+
Flavonoids	+
Volatile oils	+
Resins	-
Glycosides	+
Saponins	+
Reducing sugars	+
Anthraquinones	-

+ = present, - = absent

Table 2: Antifungal activity of the aqueous leaf extract of *Pterocarpus erinaceous*

	Diameter of zone of inhibition (mm)			
Extract/drug mg mL^{-1}	*A. niger*	*A. flavus*	*T.* sp.	*M gyp*
5	28.4	25.0	41.0	42.0
10	32.0	27.0	62.0	64.0
20	38.0	34.0	69.0	72.0
40	41.0	37.0	87.0	95.0
GS	52.0	50.0	71.0	86.0

GS = Griseofulvin, *T.* sp = *Trichophyton* specie, *M. gyp* = *Microsporum gypseum*

concentration of 40 mg mL^{-1} inhibited the growth of *Trichophyton* sp. and *Microsporum gypseum* more than the standard agent (Griseofulvin). The inhibition of moulds was less than that of the dermatophytes, this could be due to the fact that Aspergillus cause systemic infection while Trichophyton causes dermatomycoses. Most of the commonly available antifungal agents exhibit selectivity in their actions, therefore their efficacy depends on the type of infection which could be systemic or local (Schiwarz and Kaulfman, 1977).

CONCLUSION

The study has shown that the plant *Pterocarpus erinaceous* has antifungal activity on Aspergillus, Trichophyton and Microsporum. This findings lends credence to its use in the treatment of Tineases.

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