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SCREENING OF SELECTED MEDICINAL PLANTS FOR THEIR ANTIFUNGAL PROPERTIES

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ABSTRACT
Background: The rising incidence of fungal infections has created the need for the next generation of antifungal agents, as many of the currently available ones either have adverse effects, or are not active against emerging or re-emerging fungi, leading to the fast progression of resistant strains. Objectives: This study aims at evaluating the antifungal activities of some medicinal plants used traditionally for treating skin infections in Nigeria. Methods: In vitro antifungal activities of seven indigenous plants (Leptadenia hastate, Lawsonia inermis, Hyptis suaveolens, Luffa cylindrica, Jatropha curcas, Pterocarpus erinaceous and Afromaxia laxiflora) were screened against Candida albicans ATCC 10231, Candida tropicalis ATCC 13803, clinical strains of Candida albicans, Candida tropicalis, Trichophyton rubrum, Microsporum canis and Epidermophyton floccosum using agar dilution and micro broth dilution methods. Terbinafine and fluconazole were used as reference standards for comparism. Results: The results showed that the ethanol and ethyl acetate extracts of the plants produced better antifungal effects than the hexane and water extracts. Luffa cylindrica and H. suaveolens exhibited the strongest inhibitory activity against all the fungi tested with minimum inhibitory concentration values ranging between 250 and 1000 µg/mL. Conclusion: The plants screened could serve as leads for the development of new antifungal drugs.

Key words: Antifungal, agar dilution, micro broth dilution, minimum inhibitory concentration.

CRIBLAGE DE PLANTES MÉDICINALES SÉLECTIONNÉES POUR LEURS PROPRIÉTÉS ANTIFONGIQUES

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ABSTRAIT Contexte: L'incidence croissante des infections fongiques a créé la nécessité pour la prochaine génération d'agents antifongiques, comme beaucoup de ceux qui sont actuellement disponibles, soit avoir des effets indésirables, ou ne sont pas actifs contre les nouvelles ou les champignons qui refont surface, conduisant à la progression rapide de souches résistantes. Objectifs: Cette étude vise à évaluer les activités antifongiques de certaines plantes médicinales utilisées traditionnellement pour traiter les infections cutanées au Nigeria. Méthodes: l'activité in vitro antifongiques de sept plantes indigènes (Leptadenia de hastée, Lawsonia inermis, Hyptis suaveolens, Luffa cylindrica, Jatropha curcas, Pterocarpus Erinaceous et Afromaxia laxiflora) ont été projetés contre Candida albicans ATCC 10231, Candida tropicalis ATCC 13803, les souches cliniques de Candida albicans , Candida tropicalis, Trichophyton rubrum, Microsporum canis et Epidermophyton floccosum en utilisant des méthodes de dilution en gélose et de dilution au micro-bouillon. La terbinafine et le fluconazole ont été utilisés comme étalons de référence pour la comparaison. Résultats: Les résultats montrent que les extraits d'éthanol et d'acétate d'éthyle des plantes produisent de meilleurs effets antifongiques que les extraits d'hexane et d'eau. Luffa cylindrica et H. suaveolens ont présenté la plus forte activité inhibitrice contre tous les champignons testés avec des concentrations minimales inhibitrices comprises entre 250 et 1000 µg / mL. Conclusion: Les plantes examinées pourraient servir de fil conducteur à la mise au point de nouveaux médicaments antifongiques. Mots clés: Antifongique, dilution en gélose, dilution au micro-bouillon, concentration minimale inhibitrice.

INTRODUCTION

Plants have constantly played an important role in the research and development of novel anti-microbial compounds [1]. Reports from The World Health Organization show that, eighty percent of people dwelling in remote and urban regions in developing countries depend on medicinal plants for their initial health care [2].

Nigeria is home to varieties of medicinal plants many of which are used in traditional practice for the cure of skin infections like fungal infections. Fungal skin infections are common in most tribal dwellers in rural and some urban settlements where good environmental sanitation, access to portable water and general hygienic practices are lacking due to the poor socioeconomic level of the inhabitants.

The incidence of fungal infections among individuals in developing countries is on the increase (Abad *et al*., 2007). According to recent findings, resistance of some fungi to available antifungal drugs is fast becoming a major threat, especially among persons living with HIV or those on chemotherapy and drugs that suppress the immune system [3]. It is a known fact that the presently existing antifungal drugs are toxic and consequently have undesirable side effects, thus are becoming ineffective against fungi that have been in existence and upcoming ones [4].

In many countries, indigenous flora has played an important role for many generations in the treatment of infections. Based on this, it has been recognized that scientific validation of plants used to such treat infections is a viable approach in the discovery of new, effective drugs against the diseases (Taylor *et al*., 2001). Therefore, there is the need for continuous research into medicinal plants within our environment for novel antifungal compounds. In the current study, the antifungal action of hexane, ethyl acetate, ethanol and water extracts of seven medicinal plants were investigated against dermatophytes and opportunistic fungi.

MATERIALS AND METHODS

Chemicals and media

Sabouraud dextrose agar (SDA) and Sabouraud dextrose broth (SDB) were obtained from Oxoid, Germany. Dimethyl sulphoxide (DMSO), fluconazole (Cat No. F8929), Terbinafine HCl (T8826), the organic solvents *i.e.*, hexane, ethyl acetate, ethanol were obtained from Sigma Aldrich Laboratories, Germany.

Collection of plants and identification

The selection of the plants used in this research was on the basis of their ethnobotanical evidence of use for antimicrobial skin infections as documented in published literature. Fresh plants were collected from different places in Abuja. The plants were Identified and authenticated at the Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development, Abuja, Nigeria. Voucher specimens were deposited at the herbarium for reference purposes. The plants include leaves of *Leptadenia hastata* (Pers.), *Lawsonia inermis*, *Hyptis suaveolens*, *Luffa cylindrica*, *Jatropha curcas*, *Pterocarpus erinaceous* and *Afromaxia laxiflora*.

Preparation of plant extracts

Fresh plants were collected and dried in air under a shade for approximately one week. The dried leaves of the plants were then grinded in a manual mill. The powdered leaves were extracted separately by cold maceration in the various solvents (hexane, ethyl acetate, ethanol and water) for 48 h. The extracts were first sieved through a muslin cloth, and then filtered through a funnel with Whatsman No. 1 filter paper. Concentration of the filtrate was done using a rotary evaporator and dried using a water bath at 70 ^{o}C. The extracts were weighed and stored at a temperature of 4 ^{o}C until when needed.

Test fungi

The fungi used for the study include *Candida albicans* ATCC 10231, *Candida tropicalis* ATCC 13803, clinical strains of *Candida albicans, Candida tropicalis, Trichophyton rubrum*, *Microsporum canis* and *Epidermophyton floccosum* obtained from Department of Microbiology and Biotechnology, National Institute for Pharmaceutical Research and Development, Abuja, Nigeria.

Preparation/ standardization of fungi

The yeast (*Candida* sp.) was standardized by inoculating sterile normal saline solution with a 48 h pure culture by adjustment of turbidity to match 0.5 Mc Farland standard. Standardization of the dermatophytes included harvesting fungal spores from a 7 day 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 [5]. Standardization of the spore suspension to 1.0 x 10^6 spores/mL was achieved with a UV spectrophotometer (Spectronic 20D; Milton Roy Company, Pacisa, Madrid, Spain) at 530 nm (OD530) of the suspensions and adjusted to a transmittance of 70-72 %. The standardized fungal suspensions was quantified was by spreading 100 µL on Sabouraud dextrose agar plate. The plates were incubated at 37 °C for 24 h for yeast and 30 °C for 72 h for dermatophytes [6].

Antifungal assays

The antifungal activities of the extracts were achieved using micro broth dilution method according to standard reference method [1]. The stock concentration of the extracts was 8 mg/ml in 2% DMSO. The testing method involved a two-fold serial dilution of the extracts in SDB with the first well having a concentration of 4 mg/ml after inoculation with equal volume of standardized fungal suspension in SDB. Fluconazole and terbinafine hydrochloride served as positive control while 2 % DMSO served as negative control. The plates were incubated at 37 °C for 24 h for yeast and 30 °C for 72 h for dermatophytes. Minimum inhibitory concentration was seen as the lowest concentration of the extracts that inhibited fungal growth (no visible growth) after incubation period elapsed.

RESULTS

The ethno botanical uses, local names, parts used, medicinal uses and chemical constituents of the selected plants are represented in Table 1. Results on the antifungal activities of the plants are represented in Table 2. The plants exhibited variable degrees of antifungal activity. Generally, the ethyl acetate and methanol extracts of the plants were more active than the water and hexane extracts. The hexane extracts of all the plants were inactive with minimum inhibitory concentration (MIC) value greater than 8000 µg/ml.

DISCUSSION

The results of this study show that the plants exhibited greater inhibitory action on the dermatophytes than the yeasts, with *T. rubrum* and *M. canis* as the most sensitive. Dermatophytes are a specialized group of fungi that causes a zoonotic skin infection of keratinized tissues, leading to skin eruptions which last for a long time [2]. The strong activity of the ethyl acetate extracts against a broad range of fungi suggests that, most antifungal principles of these plants are soluble in ethylacetate. This observation is not strange as previous reports from our laboratory shows that, there was an increased antifungal activity of the ethyl acetate extracts over the other solvent extracts like hexane, ethanol and water (Aboh *et al*., 2014),

All the extracts of *Afromaxia laxiflora* were active against *M. canis* however, the ethyl acetate extract produced the strongest inhibitory action with an MIC of 500 µg/mL. However, the ethyl acetate extract of *A. laxiflora* produced its highest inhibition against *T. rubrum* (250µg/mL). The antimicrobial potential of *A. laxiflora* has already been expounded in literature [8]. *H. suaveolens* ethyl acetate and ethanol extracts exhibited a broad spectrum of antifungal activity. However the ethyl acetate extracts (500- 1000 µg/mL) was more effective than ethanol extract (500- 2000 µg/ml). This agrees with a work of Nantitanon et al [21], who reported the antifungal effect of ethanolic extracts of *H. suaveolens* oil which exhibited strong inhibitory action on *T. mentagrophytes* at a concentration of 10 and 20 %. The hexane and water extracts of the plant showed inhibition of all the fungal strains tested.

The ethanol and ethyl acetate extracts of *J. curcas* were effective on all of the fungal strains tested however, the ethanolic extract of the plant was most active against *C. albicans* and *T. rubrum* with MIC of 500 µg/ml. The antifungal activities of *J. curcas* have been reported by several researchers [10, 22] for example, *J. curcas* latex was reported to have inhibitory action on *C. albicans* [22]. The broad antifungal activities of *J. curcas* against the yeast and dermatophytes observed in this study, is consistent with the reports by Mbakwem – Aniebo et al [23]. These authors reported that, *J. curcas* crude stem extracts possesses a broad spectrum of antifungal effect. The poor antifungal potential of the water extracts of *J. curcas* observed in our study is also in line with the study by Sarin et al [24], where it was noted that the ethanol extract of the plant was more active than the water extract.

The ethyl acetate of *L. inermis* was most active against *C. tropicalis* with an MIC of 250 µg/mL however it inhibited the growth of all the fungal strain tested with an MIC range of 250- 1000 µg/ml. According to Arun *et al* [12], the antimicrobial activities of the plant can be attributed to the presence of flavonoids and naphthoquinones. The inhibitory action of *L. inermis* against *C. albicans* agrees with a work by Farah *et al*. (2012), although the MIC value in this study was higher. This could be attributed to the difference in extracting solvent and location of the plant. It has been reported that, the botanical and/or biological source of a medicinal plant affects its constituents as well as its physicochemical and biological/microbiological properties (Zohra *et al*., 2011; Prashant *et al*., 2011).

TABLE 1: THE ETHNOBOTANICAL USES, LOCAL NAMES, PARTS USED, MEDICINAL USES AND CHEMICAL CONSTITUENTS OF THE SELECTED MEDICINAL PLANTS

Name of plants	Local names	Part used	Medicinal uses	Chemical constituents	References
Afromaxia laxiflora (Papilionaceae) Syn. *Pericopsis laxiflora*	Makarho (Hausa) Emi (Yoruba) Osisi (Igbo)	Leaves, stem bark	Analgesic, antiparasitic, Diuretic, antibacterial	Tannin, Alkaloid, Flavonoid, Terpenoid, Saponin and Phenols	[7,8]
Hyptis suaveolens (Lamiaceae)	Misin (Gwari) Iyeye (Yoruba), Ijikara (Igbo), Tsadar lamarudu (Hausa).	Leaves, twigs, roots	stimulant, carminative, sudorific, galactogogue, parasitic infections, colic, stomachache antispasmodic, antirheumatic and antisuporific baths antiinflammatory, antifertility agents, burns, wounds, and various skin infections.	Volatile oil, starch, proteins, tannins, saponins, fats, alkaloids, glycosides	[9]
Jatropha curcas L (Euphorbiaceae)	Botuje, Lapalapa (Yoruba) Olulu-idu (Igbo), Zugu (Hausa)	Seeds, latex, leaves	Skin diseases, rheumatism, syphilis	Tannins, saponins, flavonoids, steroids, alkaloids, cardiac glycoside,terpenoid, anthraquinone.	[10]
Lawsonia inermis L. (Lythraceae)	Lalli (Igbo), Laali, Lali (Yoruba) Lalle (Hausa)	Leaves, flowers, stem bark, roots	Antioxidant, antidiabetic, hepaprotective, hypoglycemic, antimicrobial, anti-cancer, wound healing.	Flavonoids, alkaloids, tannins, Quinones.	[11,12,13]
Leptadenia hastata (Pers.). Decne. (Asclepiadaceae)	Bima (Gwari), Yaádíyaá (Hausa), Iran-aji (Yoruba) isanaje (Igbó) (Yoruba)	Leaves, latex, roots,whole plant	Hypertension, catarrh, skin diseases, wound healing, prostate complaints, aphrodisiac s	Alkaloids, saponins, phenolic glycosides, tannins, flavonoids, proanthocyanidins and triterpenes	[14,15]
Luffa cylindrica (L.) M. Roem. (Curcubitaceae) Syn. *L. aegyptiaca* Mill., *Momordica cylindrica* L	Kankan (Yoruba), Asisa (Igbo), Baska (Hausa)	Leaves, fruit, seeds		Alkaloids, flavonoids, sterols, glycosides	[16,17]
Pterocarpus erinaceus (Papilionaceae)	(banuhi (Fulani), Madubiya (Hausa), Osun dudu, Apepo, Agbelosun (Yoruba)	Leaves, root, stem bark	Fungal skin diseases e,g, athlete foot, ring worm and eczema, cough remedy, gastrointestinal upsets, chest pains, hemorrhoids, and antigonadotropic	Saponins, phenols, tannins, flavonoids	[18,19,20]

TABLE 2: MINIMUM INHIBITORY CONCENTRATION OF COLLECTED PLANT EXTRACTS AGAINST FUNGI (µG/ML)

S/N	Plants	Extracts	C.a 10231	C.a	C.t 13803	C.t	T.r	M.c	E.f
1.	*A. laxiflora*	He	-	-	-	-	-	4000	-
		Ea	1000	1000	2000	1000	250	1000	8000
		Et	8000	2000	2000	2000	1000	500	2000
		Wa	-	-	-	-	4000	2000	4000
2.	*H. suaveolens*	He	-	-	-	-	-	-	-
		Ea	1000	1000	500	500	500	1000	1000
		Et	1000	2000	1000	500	500	500	1000
		Wa	-	-	-	-	-	-	-
3.	*J. curcas*	He	-	-	-	-	-	4000	-
		Ea	500	1000	1000	1000	500	2000	4000
		Et	2000	1000	2000	1000	1000	1000	8000
		Wa	-	-	-	-	-	-	-
4.	*L. inermis*	He	-	-	-	-	-	-	-
		Ea	1000	1000	1000	250	1000	1000	500
		Et	1000	1000	500	500	500	500	500
		Wa	-	-	-	-	-	-	-
5.	*L. hastate*	He	-	-	-	-	-	-	-
		Ea	1000	1000	1000	500	1000	1000	1000
		Et	1000	1000	2000	1000	500	500	1000
		Wa	-	-	-	-	-	-	-
6.	*L. cylindrica*	He	-	-	-	-	-	-	-
		Ea	1000	1000	1000	500	250	1000	1000
		Et	1000	1000	1000	1000	500	500	1000
		Wa	-	-	-	-	-	-	-
7.	*P. erinaceus*	He	-	-	-	-	-	-	-
		Ea	1000	2000	1000	1000	1000	2000	500
		Et	2000	1000	4000	4000	2000	1000	500
		Wa	-	-	-	-	4000	4000	4000

MIC > 8000 µg/ml was taken as inactive; He - Hexane; Ea - Ethyl acetate; Et- Ethanol., Wa- water; T. r - *Trichophyton rubrum*; E. f - *E. floccosum*; M.c- *Microsporum canis*; C. a 2876 - *C. albicans* ATCC 10231; C.a- *C. albicans* (clinical isolate); C.t 13803- C. *tropicalis* ATCC 13803; C.t- *C.tropicalis* (clinical isolate)

L. hastata is one of the food plants known to possess antimicrobial activity. Several researchers have documented its antimicrobial potential [15, 26]. In the present study the plant showed a broad antifungal activity. Results from the present study revealed the broad antifungal activity of the ethyl acetate and ethanol extracts of *L. cylindrica*. The ethyl acetate fraction of the crude plant was however more active with its strongest inhibitory action against *T. rubrum* with an MIC of 250 µg/mL. The hexane and water extracts however were inactive. This disagrees with a study by Ahmad and Khan [27]; the authors reported that the n- hexane extract of the leaves showed better antimicrobial activities than the methanolic and buthanolic fractions.

The antifungal effect of the extracts of *P. erinaceus* was more pronounced on the dermatophytes than the yeast with exception of the hexane extracts which was ineffective against any of the fungi tested. The lowest MIC value (500 µg/ml) was produced by the ethyl acetate and ethanol extracts on *E. flocossum*.

CONCLUSION: The outcome of this study show that the plants investigated possess anti-fungal 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.

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