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Research Article

Anti-fungal Screening of Five Medicinal Plants used in Nigeria

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ABSTRACT

This study was specifically designed to evaluate anti-fungal properties of methanolic extracts of *Rumex acetosa*, *M.scaber*, *Senna Occidentalis*, *Lawsonia inermis* and *Spermacoce* found widely distributed in Nigeria. Three fungal organisms were tested: *Candida albicans*, *Aspergillus, and Penicillium*. Methanolic extracts of the plant materials were prepared by polarity based-solvent extraction. The anti-fungal properties were examined by the disc dilution method. Minimum inhibitory concentration (MIC) of the most potent extracts was ascertained by micro-broth dilution method The zones of inhibition pattern observed suggested that *Rumex acetosa*, *M.scaber*, *Lawsonia inermis* and *Spermacoce* were effective against *Candida albicans* with diameter (mm) of 29.50±0.29, 29.33±0.66, 29.60±0.27 and 30.49±1.66 respectively. A similar pattern was observed against *Aspergillus, and Penicillium.* However, *Senna Occidentalis* did not show any activity against all the tested organisms. The inhibitory pattern was further revealed by the MIC values. Preliminary qualitative phytochemical screening reveals the presence of alkaloids, anthraquinone, flavonoids, tannins and carotenoids.

Key words: *Rumex acetosa*, *Spermacoce*, *Aspergillus, Penicillium,* anti-fungal

INTRODUCTION

It is a well-established fact that resistance to antimicrobial agents is widespread and is a major global health problem (Lagnika *et al.,* 2016). Despite emphasis being put in research of synthetic drugs, a certain interest in medicinal plants has been developed, in part due to the fact that a lot of synthetic drugs are potentially toxic and are not free of side effects on the host (Akinpelu and Onakoya 2006). Efforts have been made to discover new antimicrobial and antioxidant compounds from various kinds of sources such as animals, plants and microorganisms in general. Starting from the human civilization, various plants had been used as medicine and as such, scientific bases need to be established.

Fungi maintains a harmful relationship with human and cause numerous pathologic conditions. The main class that causes superficial infections are: yeast, dermatophytes and moulds. Others infect subcutaneous tissues and the systemic as well (Gianluigi, *et al.,* 2020). Fungi can be directly transmitted through direct contact with infected host or indirectly through exfoliated skin or hair of tool surfaces such as combs, clothing rugs and bed linens (Farah, *et al.,* 2015). Some of the fungi that commonly affecting people include: *Candida albicans*, *Aspergillus spp*, *Fusarium spp* and *Trichophyton*.

In this study, five medicinal plants (*Rumex acetosa*, *M.scaber, Senna Occidentalis, Lawsonia inermis* and *Spermacoce*) used in Nigeria were investigated for anti-fungal activities. The plant *R. acetosa* (Sorrel in English) used for domestic remedies, and extend to complex medicinal therapies (Helena and Maria 2020). Also, *Mitracarpus scaber* is widely employed in traditional medicine in West Africa for headaches, amenorrhea, dyspepsia, hepatic diseases, veneral diseases and leprosy. Among the folkloric uses, the juice of the plant is applied topically for the treatment of skin diseases such as infectious dermatitis, eczema, and scabies (Fluck, *et al*., 1976). *Lawsonia inermis* (Henna in English) is used to treat almost any disease but specific documented uses include the treatment of fever, headache, ulcers, diarrhea, leprosy, cardiac disease, diabetes. The seed used as a deodorant and to regulate menstruation. Henna induces sleep cure headaches and bruises (Zumrutdal and Ozaslan 2012). *Spermacoce* is widely used for stomach ailments and for anti – dandruff (Vinayak, *et al*., 2013).

Preliminary phytochemical screening of the crude extracts and minimal inhibitory concentration assay were performed to ascertain the class of metabolites responsible for the anti-fungal properties.

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MATERIALS AND METHODS

Plant materials

The plant (*Rumex acetosa*, *Senna Occidentalis*, *M.scaber, Lawsonia inermis* and *Spermacoce*) sample were collected at Jere local government, Borno state and around Oshilaja Street, Ibadan, Oyo State. The plants were identified at the department of Botany, university of Maiduguri, Borno state.

Preparation of extracts

The extracts were prepared as described by Ayankunle, *et al*., 2012. The root bark of *Lawsonia inermis* and *Senna Occidentalis*, leave bark of *Rumex acetosa* and fresh leaves of *M.scaber* were washed thoroughly with distilled water and allowed to dry at room temperature. The dried samples were chopped into small pieces using a pestle and mortar and were pulverized into powdery form using an electric blender. Various samples (100 g) of the powdered air-dried plant materials were soaked in 98 % methanol (1000 ml) and left on a shaker (IKA KS260 Basic) for 24 hours. At the end of the extraction, the suspensions were filtered using Whatman filter paper (Whatman international limited, Maidstone, England). The filtrates were then dried to get the concentrated extracts. These extracts were thereafter stored at 4^0C prior to usage.

Qualitative Phytochemical Screening

The extracts were screened for phytochemicals following scientifically certified methods for various secondary metabolites such as alkaloids, anthraquinone, flavonoids, tannins and carotenoids.

Test organisms

Pure clinical isolates of *Candida albicans, Aspergillus, and Penicillium* were obtained from the Laboratory Department (Parasitology), University of Maiduguri Teaching Hospital (UMTH). The organisms were grown on a Mueller Hinton Agar in an incubator at 37ºC for 24 hours. The antifungal activities of the plant extracts were measured by culture media using Mueller Hinton Agar and Chocolate Agar.

Anti-fungal activity assay and Minimum Inhibitory Concentration (MIC)

The anti-fungal was done using the punch well method described by Stokes, 1975. Briefly, the plates were prepared by dispensing 20 ml of nutrient agar into sterile Petri plates and allowed to set. A 4 mm cork borer was used to punch holes in the medium. Four wells were made on each Petri plate, adequately spaced out after inoculation. About 0.2 ml of the different concentrations of the extracts was introduced into each well. The petri plates were incubated at a temperature of 37 0C for 24 hours for *C. albicans* while for the remaining organisms one plate was used for each organism and were prepared by dispensing 20ml of SDA and incubated at a temperature of 37 0C for 168 hours, after which observation for the zones of inhibition was conducted. Measurement of the zones of inhibition was carried out. A standard anti-fungal (Fluconazole) was used as positive control. The MIC was then identified accordingly.

Statistical Analysis

The results were expressed as mean ± SEM of the studied organisms using the analysis of variance test (one way ANOVA), followed by statistical package for the social science software (SPSS). Values of $P < 0.05$ considered significant.

RESULTS

Table 1 reveals the result of the phytochemical screening. It shows that *M. scaber* poses all the secondary metabolites screened. The plants *Rumex acetosa* and *Spermacoce* shows the absence of carotenoids while *Lawsonia inermis* reveals the absence of both alkaloids and carotenoids. It was revealed that *Senna Occidentalis* lacks anthraquinone.

Table 2 shows the zones of inhibition exhibited by various plant extracts with *Spermacoce* as the most effective against *Candidas albicans* (30.49±1.66).

Table 3 reveals the MIC values for the plant extracts with *Rumex acetosa* and *Spermacoce* most effective against *Candidas albicans* (50mg/ml). *M. scaber* was shown to be the most effective against *penicillium.*

DISCUSSION

This study focuses on antifungal efficacy of *Rumex acetosa*, *Senna Occidentalis, M.scaber, Lawsonia inermis* and *Spermacoce* against *Candidas albicans, penicillium* and *Aspergillus spp*, were tested. This is on the bases that the plants are known traditionally for their various medicinal uses in Nigeria. The investigation into these medicinal plants showed that all the extracts with the exception of *S. occidentalis* root bark were found to be potent and exhibited a dose dependent antifungal activity. The extracts of *Spermacoce* inhibited *Candida albicans* and *penicillium* to a significant level while minimal inhibition was observed on *Aspergillus spp*. as revealed by the zone of inhibitions (Table 2). It was observed that the leave bark extract of *R. acetosa*. Inhibited all the three organisms tested (Table 2). Also, the leave extract of *M.scaber* inhibited all the organisms tested and showing a near similar zones of inhibition as revealed (Table 2). However, the root bark extract of *L. inermis* shows some degree of inhibition of *Candida albicans* and *penicillium*, and no inhibition pattern against *Aspergillus spp.* Observed (Table 2). The methanolic root bark extract of *S. occidentalis* didn't inhibit any of the tested organisms (Table 2). The plant *R. acetosa* has MIC value of 50mg/ml against *Candida albicans* and *Aspergillus spp* which indicates effectiveness of the plant. The least effectiveness was observed with the plant *Lawsonia inermis* (Table 3).

Based on the qualitative phytochemical studies, the plants were observed to contain biologically active important compounds. Anthraquinone was present in all the plants tested with the exception of *S. occidentalis* (Table 1). This could be the possible contributing factor to the ineffective nature of *S. occidentalis* against the organisms tested. Plant – derived anthraquinones are known for their effectiveness against fungi (Wuthi-udomlert and Gritsanapan 2010). It was observed that the plant *M.scaber* contains all the plant secondary metabolites screened (Table 1). Also, *R. acetosa* was observed to contain

Table 2: Phytochemical screening of methanol Extract of the plants

Plant name	Alkaloid	Anthraquinone	flavonoids	tannins	Carotenoids
Rumex acetosa	+	+	+	+	-
M. scaber	+	+	+	+	+
Spermacoce	+	+	+	+	-
lawsonia inermis	-	+	+	+	-
Senna occidentalis	+	-	+	+	-

Key: + = Presence of the phytochemical: - = Absence of the phytochemical.

Table 3: Anti-fungi potential of selected Medicinal Plants against Test Organisms. Extract concentration is 250 mg/ml

Plants name	part used	Diameter of the zone of inhibition (mm) against test organisms		
		C.albicans	*Penicillium*	*Aspergillus spp*
R. acetosa	leave bark	29.50±0.29[a]	26.00±0.58[a]	24.17±0.00[a]
M. scaber	Leave	29.33±0.66[a]	25.50±0.28[a]	27.50±0.28[a]
Spermacoce	leaves	30.49±1.66[a]	15.03±0.26[a]	26.06±026[a]
L. inermis	root bark	29.60±0.27[a]	14.32±0.26	---
S. occidentalis	root bark	---	---	---
Control (Fluconazole)		28.17±0.60[a]	26.17±0.73[a]	24.67±0.58[a]

Values are expressed as Mean ± SEM (mm) of triplicate determinations: [a] Superscript indicates significantly different (P<0.05).

Table 3: MIC of the Plant Extracts against Test Organisms

Plant Extracts/ Positive control	MIC (mg/ml)		
	C.albicans	*Penicillium*	*Aspergillus spp*
R. acetosa	50	500	50
M. scaber	500	50	50
Spermacoce	50	500	500
L. inermis	500	500	-
Fluconazole (p. control)	5	2.5	2.5

− Represents not tested; MIC – minimal inhibitory concentration; p. control implies positive control.

all the metabolites screened with the exception of carotenoids (Table 1). Bello, *et al.*, 2019 reported a similar observation while screening for active components of *R. acetosa*. Previously, *M.scaber*, *Lawsonia inermis* and *Spermacoce* were reported to contain similar secondary metabolites (Ali, 2019; Namadina, *et al*., 2020).

Previous evidence shows that the extract of *R. acetosa* has some inhibitory activities on the adhesion of *C. albicans* to buccal epithelial cells (Susana, *et al*., 2008). It has also been reported that the fruit extract of *R. acetosa* has effect against *Aspergillus spp*. (Magdalena, *et al*., 2011). The plant *M.scaber* was previously found to be effective against *C. albicans* and *Aspergillus spp* (Cimanga et al., 2004). The seeds of *Spermacoce hispida* was previously found be to potent against *C. albicans* and *Aspergillus spp* (Rajkumar, 2013). The results of this study are in conformity with previous studies conducted on the plants with some minor variations due to some factors such as geographical distributions, parts of the plant used in different studies and the solvents used for the plant extraction.

Conclusion

The inhibitory pattern observed in this study could serve as scientific evidence for the use of these plants against some pathogenic human fungi by some people in both the urban and rural part of Nigeria. It is however important to further validate the potency of these plant through a standard bio – guided assay to know and isolate the plant constituents responsible for the biological activities. Also, the acute and sub – acute toxicity study of the isolated compounds as well as the crude extracts could establish an appropriate dosage to be used.

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