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Original Research Article

Phytochemical screening, Antioxidant and Anti Fungal activities of Certain Sudanese Medicinal Plants against *Tinea capitis*

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Abstract

Herbal medicines have been widely utilized as effective remedies for the prevention and treatment of multiple health conditions for centuries by almost every known culture. A significant fraction of the drugs are either natural products or derived from natural products. This study investigates the antifungal activity of *Lawsonia inermis, Aloe vera* and *Senna alata* against *Tinea capitis*. *Tinea capitis* is a superficial fungal infection (ring worm) that causes hair fall in specific parts of the head. The main objective of this study was to compare between the antimicrobial effects of the plants extracts on two *Tinea capitis* subspecies, as well as to detect the phytochemical constituents of each and their antioxidant activity. Fungal strains were isolated from patients and identified using microscopical examination and biochemical tests. All three samples were extracted with ethanol 96% and phytochemical constituents were detected using qualitative standard methods. Anti-microbial activity of three samples extracts with different concentrations was determined using Disc diffusion method and the inhibition zones were measured. The three plants were also screened for their antioxidant activity and gave positive results against the *T.capitis* species provided (*T.verrucosm* and *T.rubrum*). *S.alata* showed the highest activity against two species with slight difference followed by the L.*inermis* and *A.vera* respectively compared with the standard drug fluconazole.

Keyword: *Tinea capitis*, antifungal activity, antioxidant activity, Sudan, *Lawsonia inermis*, *Aloe vera*, *Senna alata*.

INTRODUCTION

Herbal medicine is the study of herbs and their medicinal uses. This can be extended to include the cultivation, collection, or dispensing of aromatic plants, especially those considered to have medicinal properties (Ameh et al., 2010). Herbalism, as a practice, has been around for centuries. It takes different forms, using different plants and herbs to be used in different ways. Herbal medicines can be used to treat a disease, relieve symptoms or as a supplement to aid the patient's recovery process. Plants have been the basis for medical treatments through much of human history, and such traditional medicine is still widely practiced today (Skipper, 2007). The plant *Lawsonia inermis* belongs to family Lythraceae, It is much branched, deciduous, glabrous, sometime spine scent shrub or small tree with grayish brown bark, attaining a height of 2.4-5 m. It native to tropical and subtropical regions of Africa, southern Asia, and northern Australia in semi-arid zone and oases in the Sahara. *L.*

inermis leaves possess Antioxidant, anti diabetic, hypoglycemic, antimicrobial, anticancer and wound healing properties (Abid et al., 2005). *Aloe vera* belongs to family Liliaceae, *A. vera* is a stem less succulent plant growin to 60-100cm (24-39 in) tall, spreading by offsets. The leaves are thick and fleshy, green to grey-green with some varieties showing white flecks on the upper and lower stem surfaces. It is indigenous to Africa and Mediterranean countries. It is reported to grow wild in the islands of Cyprus, Malta, Sicily, Canary cape, Cape Verde and arid tracts of India. *Aloe vera* has been used externally to treat various skin conditions such as cuts, burns and eczema. *Aloe vera* gel is useful for dry skin conditions, especially eczema around the eyes and sensitive facial skin (Vogler and Ernst, 1999). Useful in various diseases such as type II diabetes, arthritis, eye disease, tumor, spleen enlargement, liver complaints, vomiting, bronchitis, asthma, jaundice and ulcers. Relieves constipation, maintains a good gastric pH, and helps in inflammatory bowel diseases, non-ulcer dyspepsia, gastric and duodenal ulcers. A dietary supplement in pre and post-operative patients, postmenopausal women and in cases of osteoporosis (Barcroft, 2003). *Senna alata* belong to family Fabaceae, is a shrub with usually an average height of between 1 and 5metres and has horizontally spread branches. Its leaves are par pinnate of between 30 to 60 cm long and consisting of 8 to 20 pairs of leaflets (Farnsworth and Bunyapraphatsara, 1992). *S. alata* (*Cassia alata*) is an ornamental flowering plant native to the Amazon Rainforest (Springer-Verlag, 2007). Due to its beauty, it has cultivated in tropical Africa, tropical Asia, Australia, Mexico, the Caribbean islands, Melanesia, Polynesia, Hawaii and India,West Bengal, and Andhra. It grows well in forested areas of West Africa. In Indonesia, Philippines and Thailand, this plant can be found all over the countries, sometimes cultivated for medicinal purposes (Bansiddhi and Pecharaply, 1988). Leaf extract of *S.alata* is credited for the treatment of constipation, inguinal hernia, intestinal parasitosis, syphilis and diabetes (Chatterjee et al., 2012). The juice of the fresh leaf of the plant is universally recognized as a remedy for parasitic skin diseases, and is used in many and pustular skin infections by simply rubbing the crushed leaves alone or mixed with lime juice or oil. The juice expressed from the fresh leaves is taken along with lime juice for worms. The bark has been recommended as a tanning material. The juice of the root is rubbed into cuts for tattooing or tribal markings (Darziel, 1937). The leaves of *S.alata* have been reported to be useful in treating convulsion, onorrhea, heart failure, abdominal pains, oedema and it's also used as a purgative (Laxative activity of Cassia alata, 1993). Skin problems treated with *S.alata* include ringworm, favus and other mycoses, impetigo, syphilis sores, psoriasis, herpes, chronic lichenplanus, scabies, shingles, eczema, rash and itching. In veterinary medicine too, a range of skin problems in livestock is treated with leaf decoctions. It is also used for treating digestive tract infections, intestinal worms, typhoid fever, poison, hepatitis, yellow fever, wounds and viral infections .The utilization of plants against diseases such as cancer, parasitic infection, rheumatism, arthritis, wound treatment, tumor growth, stroke, jaundice, typhoid, fibroid, syphilis and gonorrhea have been well documented (Okpuzor et al., 2009). *Tinea capitis* is being widely distributed in Sudan and with its easy transmission, has been a great problem and knowing that only antifungal tablets are available with its known side effects, herbal treatment with *L. inermis, S. alata* and *A. vera* are now the best option even economically with far less side effects. Phytochemical screening of the components of herbal remedies is essential in identifying the active compounds.

Tinea capitis in Sudan

A number of school children were examined for ringworm infection and the casual organisms were identified by culture method. Certain areas were not visited due to transport difficulties and for the same reason towns rather than villages were visited. A rural survey might have changed the picture, because in general, rural ringworm tends to be from animal origin and does not occurs in epidemics, whereas urban ringworm is caused by Anthropophilic species and can occur in epidemics. The result revealed an overall incidence of ringworm infection of 4.2% rising to 17%. *Tinea capitis* presented the main problem 3.2% having this infection. Fungi were isolated from 172 scalp specimens; 79 (46%) were *M.audouinii*,75 (43.6%) *T.violaceum*,13 (7.5%) *T.soudanense* and 5 (2.9%) *M.canis* (Mahgoub, 1968).

MATERIALS AND METHODS

Materials

Plant Materials

The plants *Lawsonia inermis*, *Aloe vera* and *Senna alata* were collected from a herbalist in the local market in Khartoum. The plant were identified and authenticated at the Aromatic and Medicinal Plant Institute – Research Center. The voucher specimens were deposited at the pharmacognosy department, Faculty of Pharmacy at the University of Medical Sciences and Technology.

Isolated fungal strains

Two fungal species were used in this study. The species used in this experiment were isolated from patient in Ultra

lab in Mac Nimir Street, cultured and identified using standard procedure.

Culture media

Sabouraud Dextrose Agar

Sixty two grams of the powdered Sabouraud dextrose agar, was weighed, dispersed in 1 ml water and allowed to soak for 10 min, swirled to mix then sterilized by autoclaving for 15 min at 121 ^{o}C, cooled to 47 ^{o}C, mixed well and then poured in to sterile Petri dishes.

METHODS

Preparation of the sample extracts

Leaves from *Lawsonia inermis , Aloe vera* and *Senna alata* were cleaned ,air dried and ground to powder using a pestle and mortar. The samples 60.92 grams, 20.32 grams and 48.67 grams of powder respectively were added to conical flasks separately and extracted with ethanol 96% at room temperature for 3 days. The extracts were filtered using whattman number 4 filter paper. After filtration the extracts were concentrated under reduced pressure.

Preparation of fungal strains samples

Suspected lesions were cleaned with 70% ethyl alcohol to remove any dirt and contaminating bacteria. Skin scales and crusts were collected from the erythematous, peripheral, actively growing margins of the lesions by scraping across the inflamed margin of the lesion into the apparently healthy tissue using the blunt edge of a sterile surgical blade onto clean glass slides. Hair specimens were collected by using epilating forceps to pluck along the base of the hair shaft, and scales were scraped from the surface using the blunt edge of a sterile surgical blade. The cutting of hair was avoided as the infection is usually confined to the root, very near the scalp's surface. Specimens were collected and sealed in sterile dry Petri dishes and labeled with the patient's name, age, sex, date of collection, and site of infection and subsequently brought to the laboratory for mycological examination. The samples were divided into two portions: one for microscopic examination and one for culture (Farnsworth and Bunyapraphatsara, 1992).

Microscopic examination and fungal culture

For direct microscopy, the samples collected were screened for the presence of fungal elements using a 10% KOH with 40% Dimethylesulphoxide (DMSO) mount mixed in equal proportion. Two to three drops of the KOH+DMSO mixture were kept on a clean, grease-free glass slide. The sample (skin scraping or hair plucking) was placed in the KOH+DMSO drops on the slide, and a clean cover slip was placed on the sample and pressed to prevent the formation of air bubbles. The sample was kept in KOH +DMSO and then observed after 5–8 minutes. DMSO increases the sensitivity of the preparation and softens keratin more quickly than KOH alone in the absence of heat. Figure 1

Phytochemical Screening of *Lawsonia inermis, Aloe vera* and *Senna alata*

The plant samples of the three plants were extracted ethanol 96% and the prepared extracts were used for detection of different secondary metabolites according to standard methods (Trease and Evans, 2002).

Antifungal activity

Disc Diffusion Method

Disc diffusion method for antimicrobial susceptibility testing was used as standard method to assess the antifungal activity of the plant extracts. A fungi culture which has been adjusted to Sabouraud agar Plates evenly using a sterile swab. The plates were dried for 15 minutes and then used for the sensitivity test. The discs impregnated with a series of plant extracts were placed on the Sabouraud dextrose agar surface (Guerin-Faublee et al., 1996).

Minimum Inhibition Concentration (MIC) Determination

MIC was determined using Inhibitory Concentrations in Diffusion method (Bauer et al., 1966). It was done by carrying out the diffusion test with four different concentrations of the plant extracts. The lowest concentrations that inhibit the growth of fungi was noted and considered as the MIC value for each of the fungal strain**.**

Antioxidant activity

DPPH radical scavenging activity

Antioxidant activity of the extracts was estimated using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) method (Oyaizu, 1986). Test samples were dissolved separately in 5% DMSO to get test solution of 1 mg/ml. Assay was

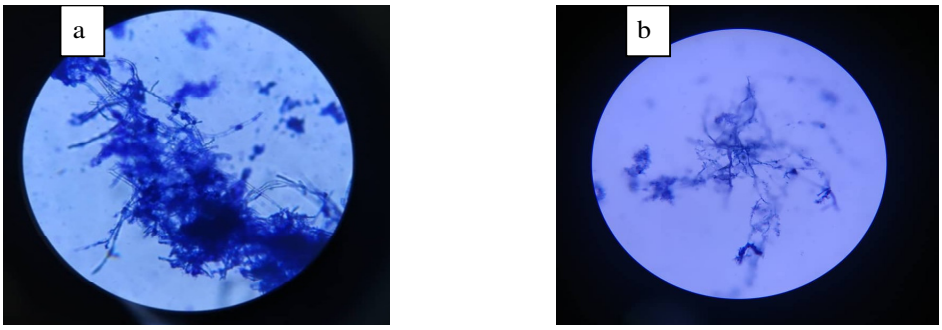

Figure 1. Microscopic examination of two species of fungia) *Tricophytone verrucosum*; b) *Tricophytone rubrum*

Table 1. Phytochemical Screening of *L.inermis* ,*A.vera* and *S.alata* extracts

Test		Sample		
		Lawsonia inermis	***Aloe vera***	***Senna alata***
Alkaloids	Wagner	+ve	+ve	+ve
	Dragendorffs	-ve	-ve	+ve
	Mayer	+ve	+ve	+ve
Flavonoids	Lead acetate	+ve	+ve	+ve
Sterols	Salkowski	+ve	+ve	+ve
	Liberman-burchard	+ve	+ve	+ve
Triterpinres	Salkowski	+ve	+ve	+ve
	Liberman-burchard	+ve	+ve	+ve
Tannins	Ferric chloride	+ve	+ve	+ve
	Gelatin	+ve	+ve	+ve
Saponins	Frothing test	-ve	+ve	+ve
Glycosides	Kellar killani	+ve	+ve	+ve
	Kedd's test	+ve	+ve	+ve
Anthraquinones	Ammonia test	-ve	+ve	+ve
Reducing sugar	Fehling's test	+ve	+ve	-ve

performed in 96-well, microtiter plates. 140µl of 0.6 x 10-6mol/l DPPH will be added to each well containing 70 µl of sample. The mixture was shaken gently and left to stand for 30 min in dark at room temperature. The absorbance was measured spectrophotometrically at 517 nm using a microtiter plate reader.

RESULTS AND DISCUSSIONS

Phytochemistry

Yield percentage

L.inermis gave the highest percentage of extractive yield (8.99%), followed by *S.alata* leaves (7.13%) and *A.vera* (4.05%).

Phytochemical screening

The qualitative phytochemical analysis was performed initially with different chemical reagents to detect the nature of phytoconstituents and their presence in *L.inermis*, *A.vera* and *S.alata* leaf extracts and results were presented in Table (1). Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, sterol/triterpenes, tannins and glycosides. *A.vera* and *S.alata* revealed presence of saponins which in *L.inermis* were not detected. Sugars were detected in all samples except *S.alata*. Results were compared with published data in the current literature and differences may be due to geographic location (18,19,20).

Biological activity

Antifungal activity of plants extracts against *Tinea* species

Occurrence of dermatophytic infection is a public health problem especially in children. This is because of the development of antifungal drug resistance of the pathogens and side effects exhibited by the drugs used

Table 2. Antifungal activity against *T.verrucosum* and *T.rubrum* of *L.inermis*

Sample	Concentration	Diameter of Inhibition Zone (mm)	
		T.verrucosum	***T.rubrum***
Lawsonia inermis	100 mg/ml	28	27
	50 mg/ml	20	22
	25 mg/ml	10	17
	12.5 mg/ml	-	13
	6.25 mg/ml	-	-
Aloe vera	100 mg/ml	18	23
	50 mg/ml	17	16
	25 mg/ml	11	14
	12.5 mg/ml	-	10
	6.25 mg/ml	-	-
Senna alata	100 mg/ml	30	30
	50 mg/ml	24	17
	25 mg/ml	12	16
	12.5 mg/ml	9	10
	6.25 mg/ml	-	-
Fluconazole	5 mcg	13	9

Table 3. MIC for *L.inermis*, *A.vera* and *S.alata*

Sample	MIC (mg/ml)	
	T.verrucosum	***T.rubrum***
L.inermis	25	12.5
A.vera	25	12.5
S.alata	12.5	12.5

Table 4. DPPH radical scavenging activity of plants extracts

Sample	***L.inermis***	***A.vera***	***S.alata***
DPPH%	34%	Not detected	60%

for fungal diseases (Sagar and Vidyasagar, 2013). The antifungal activity at concentrations 100, 50, 25, 12.5 and 6.25 mg/ml of *L.inermis*, *A.vera* and *S.alata* ethanolic extracts was determined against two isolates fungi using disc diffusion method. Results were presented in Tables 2, 3 and 4. All three sample extracts showed high activity against the two isolated fungi. The *S.alata* leaf extract showed the highest activity against *T. verrucosum* and *T. rubrum* at concentration 100 mg/ml with inhibition zone 30 mm, followed by *L.inermis* with inhibition zone 28 and 27 mm respectively, while the *A.vera* extract showed the lowest activity against *T. verrucosum* and *T. rubrum* with inhibition zone 18 and 23 mm respectively. Ethanolic extract of the different plant species reduced colony growth of the two test dermatophytes. The fungitoxic effects of ethanolic extracts of most plant species tested in the present work indicate importance of many plant species as a natural source of antimycotic substances (Sagar and Vidyasagar, 2013). In the present investigation, *L. inermis* exhibited antifungal species against two fungi as was also shown by Singh and Pandey (Singh, 1989) and Sagar (Sagar and Vidyasagar, 2013). The present study indicates that the majority of the plants tested are an important source of anti fungal compounds that may provide renewable sources of useful antifungal drugs against dermatophytic infections in humans.

The response of dermatophytes to treatment with various plants extracts varied from organism to organism, nevertheless it was shown to be concentration dependent as greater inhibition of growth was observed as the concentrations of the extracts increased. As all the plants investigated in the present work are common in Sudan, the recovery of their compounds is high and thus, these species may be exploited as potent herbal chemotherapeutics for dermatomycosis. Phyto-constituents such as alkaloids, flavonoids, tannins, phenols ,saponins and other aromatic compounds in the plants serve a defense mechanism against predicted microrganisms, insects and herbivores (Shihabudeen et al., 2010).

Minimum inhibitory concentration

The best MIC against *T.verrucosum* showed in *S.alata* leaf extract (12.5mg/ml) followed by the other samples (25mg/ml),while all plant sample extracts showed MIC against *T.rubrum* with (12.5mg/ml).

Antioxidant activity

DPPH radical scavenging assay is the most common method used in the study of antioxidant activity of plant extracts. It results in the formation of stable free radical which can be detected by common spectrophotometric technique. Decrease in absorbance shows the more efficient antioxidant activity of the extraction terms of hydrogen atom donating capacity (Blios, 1958). Several epidemiological studies suggest that plants rich in antioxidants play a protective role in the health and against diseases and their consumption lowered the risk of cancer, heart diseases, hypertension and stroke. The curative properties of medicinal plants are due to the presence of different phytoconstituents such as alkaloids, flavonoids, glycosides, phenols, saponins, sterols and others (Lalnuridanga and Lalrinkima, 2012). Antioxidant activity of the ethanolic leaf extracts of *L.inermis*, *A.vera* and *S.alata* was evaluated using DPPH assay. The highest result was shown by *S.alata* (60%) followed by *L.inermis* (34%) and void in *A.vera*.

CONCLUSIONS

Dermatophytic infection is a common infection that constitutes public health problem among children. Anti dermatophytic activity of ethanolic extracts of *L.inermis, A.vera* and *S.alata* was investigated against isolates of dermatophytic fungi. The three plants have shown great effect on the *Tinea capitis* species specially subspecies *Trichophyton rubrum*. The *S.alata* is now one of the most widely used plant for this disease as it's the first most effective and almost found in all Sudan with cheap price, followed by the *L.inermis* which is used even for other skin diseases and *A.vera* comes last which is a useful remedy for facial and skin conditions.

Conflict of interests

Authors declare no conflict of interests.

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