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ANTIDERMATOPHYTIC ACTIVITY IN CRUDE EXTRACTS OF SOME MEDICINAL PLANTS OF JALPAIGURI DISTRICT

Pushpanjali Ray

Department of Botany

Ananda Chandra College, Jalpaiguri

West Bengal, India

Suman Sen

Department of Botany

Ananda Chandra College, Jalpaiguri,

West Bengal, India

Rita Som Paul

Department of Botany

Siliguri College

Siliguri, West Bengal, India

Abstract— Thirteen commonly found species of plants reported to be used against skin ailments, were selected for the present study. The aqueous leaf extracts were first screened qualitatively for the presence of nine biochemical components (alkaloids, anthranol glycosides, saponins, phenols, tannins, flavonoids, terpenoids, protein and reducing sugar) and the scores were noted. All the extracts were further tested by agar incorporation method against three common dermatophytes – *Trichophyton rubrum*, *Trichophyton mentagrophytes* and *Microsporum audouinii*. The extracts of *Catharanthus roseus* and *Cassia alata* were found to be the most potent followed by *Ageratum conyzoides*

Keywords— antidermatophytic, aqueous extract, biochemical, agar incorporation

I. INTRODUCTION

It is well known that all the plants have some or the other medicinal properties. However, most of the research both in India and abroad is now focused on extraction of active principles in order to develop novel drugs, which is very costly.

The dermatophytes are a group of fungi having ability to invade keratinized tissue like skin, hair, and nail of animals and human beings leading to an infection. These are commonly referred to as ringworms. The etiologic agents of dermatophytoses are classified in three anamorphic (asexual or imperfect) genera, *Epidermophyton*, *Microsporum*, and *Trichophyton*, of anamorphic class Hyphomycetes of the Deuteromycota commonly known as Fungi Imperfecti [1]. Superficial fungal infections or tinea infections are common skin diseases, affecting millions of people worldwide. Dermatophytes are unique among fungi in that they cause communicable diseases. Earlier, most dermatophyte strains

had relatively restricted geographical distribution. However recently, dermatophytosis has become one of the most common human infectious diseases in the world and is cosmopolitan in distribution [2]. Treatment of these usually demands the use of the antifungal agents such as griseofulvin and amphotericin. Steroids (ketoconazole, oxyconazole, econazole, etc.) are also being used at present for treatment of mycoses, according to Weinstein and Berman (2002) [3], who also suggest caution and careful diagnosis while using the steroid antibiotics.

Fungi are difficult to control due to their lifestyle and etiology. Besides, the problem of resistant dermatophytes is not new [4]. The lack of new antifungal agents, the apparent increase of the infections by the dermatophytes and the emergence of the strains resistant to the antifungal therapy demands the exploration into natural products [5].

Traditional medicines are very promising for treatment of dermatomycoses especially in tropical developing countries, including India. It is in this context that the people use several plant derived preparations to cure skin diseases as well. Even the technologically advanced countries also rely upon the traditional medicine derived from plants [6].

Recently, antidermatophytic studies have been done with the active principles of numerous medicinal plants. Antidermatophytic activity of dichloromethane and methanol extracts of whole plant of *Allamanda cathartica* was evaluated against two pathogenic dermatophytes *Trichophyton rubrum* and *Microsporum gypseum* by Nahar *et al* (2010) [7]. Adejumo and Bamidele (2009) [8] studied antidermatophytic potential of six medicinal plant extracts against *T. rubrum* and *T. mentagrophytes*. The simple preparations made from the common plants like *Cassia sophora* L, *Leucas aspera* (Willd) Link, *Urena lobata* L. and *Clerodendrum indicum* (L) Kuntze have been described in detail by Hanif *et al* (2009) [9]. Sagar



and Vidyasagar (2013) [10] described the antidermatophytic activity of *Cassia occidentalis*, *Cassia tora*, *Lawsonia inermis*, *Caesalpinia bonducella*, *Xanthium strumarium* against *Trichophyton tonsurans*, *T. mentagrophyte*, *T. rubrum*, *Microsporum gypseum* and *Epidermophyton floccosum* prepared in different solvent system namely methanol, alcohol, acetone, petroleum ether, chloroform and ethyl acetate.

The extraction of active principles also has lead to indiscriminate use and eradication of the valuable medicinal plants. Research into the use of commonly found herbs in their raw or easily prepared form, is lacking. Besides, out of all the ailments, dermatomycoses have not received much attention due to their non-lethal action. Other diseases like cancer, tuberculosis, typhoid, etc get attention and are being investigated for novel treatments due to their deadly nature.

However, very few studies have been done with aqualous crude extracts presumably due to difficulty in handling and preserving the extracts. This topic needs urgent attention.

The present investigation throws some light on the use of some lesser known medicinal plants in aqueous extract form against the dermatophytes. Based on the information obtained from independent survey by the authors [11] and other literature [12,13], thirteen commonly found plants reported to be used against skin diseases, were chosen for the present study. The preparations were also analysed biochemically for the presence of active ingredients.

II. MATERIALS AND METHODS

A. Plant Material

Thirteen plant species as given in Table 1, were collected, dried, mounted on the herbarium sheets as per the protocol of Jain and Rao (1977) [14] and identified using the standard literature and by comparing with the herbarium specimen maintained at Taxonomy and Environmental Biology Laboratory of the Department of Botany, University of North Bengal. The reference specimen were deposited in the Herbarium of Botany Department, A. C. College, Jalpaiguri and Taxonomy and Environmental Biology Laboratory, University of North Bengal. For nomenclatural treatment and family delimitation www.theplantlist.org has been followed. Separate beds of these plant species were prepared and maintained in uniform natural conditions at the Medicinal Plants Garden of Ananda Chandra College, Jalpaiguri, for the purpose. Adequate watering and use of organic manure (cowdung and vermicompost) once in six months was followed for maintenance. Nimacin spray was used as insecticide.

B. Fungal Isolates

Trichophyton rubrum (MTCC No 296), *Trichophyton mentagrophytes* (MTCC No7687) and *Microsporum audouinii* (MTCC No 8197) strains obtained from Microbial Type

Culture Collection, Chandigarh, were used in the present studies.

Table 1. Specimen identified and deposited at NBU Herbarium, University of North Bengal

Sl No	Name	Family	NBU accession No.
1.	<i>Ageratum conyzoides</i> (L.) L.	Asteraceae	10519
2.	<i>Elephantopus scaber</i> L.	Asteraceae	10520
3.	<i>Cassia alata</i> L.	Fabaceae	10521
4.	<i>Heliotropium indicum</i> L.	Boraginaceae	10522
5.	<i>Artemisia vulgaris</i> L.	Asteraceae	10523
6.	<i>Cannabis sativa</i> L.	Cannabaceae	10524
7.	<i>Leucas zeylanica</i> (L.) W.T. Aiton	Lamiaceae	10525
8.	<i>Phlogacanthus thyrsoformis</i> (Roxb. ex Hardw.) Mabb.	Acanthaceae	10526
9.	<i>Sphagneticola calendulacea</i> (L.) Pruski.	Asteraceae	10527
10.	<i>Catharanthus roseus</i> (L.) G. Don	Apocyanaceae	10528
11.	<i>Andrographis paniculata</i> (Burm.f.) Nees.	Acanthaceae	10529
12.	<i>Eclipta prostrata</i> (L.) L.	Asteraceae	10530
13.	<i>Justicia adhatoda</i> L.	Acanthaceae	10531

C. Maintenance of isolates

The dermatophytes were maintained on Sabouraud Dextrose Agar (SDA) (Hi Media) medium and Potato Dextrose agar Medium (PDA) at 25-30 °C. These were subcultured every month and examined microscopically for their purity.

D. Preparation of aquaous extract

The freshly collected leaves of the thirteen plant species were brought to the laboratory, washed in running tap water and blotted dry. The experiments were conducted in November to March of 2011-12 and 2012-13.

Ten grams of fresh leaves were taken in a sterile container and rinsed in sterile distilled water. After draining the water, 0.1% Mercuric chloride (Qualigens) solution was added and surface sterilized for 1 minute. Material was rinsed repeatedly (3-4 times) with sterile distilled water to remove all the traces of mercuric chloride. The leaves were blotted dry with sterile blotting paper and crushed in sterile mortar and pestle with 15 ml of sterile distilled water. It was then centrifuged in sterile centrifuge tube at 5000rpm for 10 minutes. The pellet was discarded, while supernatant was saved for future use. The



extract was immediately used for biochemical characterization and antifungal bioassay. The preparations were evaporated to dryness and the amount of the solids extracted were weighed in order to determine the concentration (mg per ml). Prior to the biochemical and antifungal assays the concentration of extract was adjusted to 10mg/ml and 15 mg/ml.

E. Phytochemical screening

The qualitative phytochemical evaluation of the plant extracts was carried out immediately after extraction as per the methodology of Brain & Turner (1975)[15] using the extract of 15mg/ml concentration. For negative control, water was used instead of extract. Positive control was also used for reference in each test separately.

Alkaloids: Plant extract was dissolved in dilute hydrochloric acid and filtered. The filtrate was subjected to Mayer's test to detect alkaloids. Mayer's reagent (Potassium Mercuric Iodide) was added to the filtrate. Formation of yellow cream precipitate confirms presence of alkaloids. Quinine sulphate was used as positive control.

Anthranol glycosides: Plant extracts hydrolyzed with dilute hydrochloric acid was subjected to Modified Borntragers test. To the extract, Ferric chloride solution was added and immersed in boiling water for 5 minutes. The mixture was then cooled and shaken with an equal volume of Benzene. The Benzene layer was separated and treated with ammonia solution. Formation of rose pink colour in the ammoniacal layer indicated presence of anthranol glycosides. Hydroquinone was used as a positive control.

Saponins: Froth test was done, where extract was dissolved in 20ml distilled water and shaken in a graduated cylinder for 15 minutes. Formation of 1 cm foam layer confirms presence of saponins. Pea powder was used as a positive control.

Phenols: Ferric chloride test was done. To the extract, few drops of ferric chloride were added. Bluish black colour confirmed presence of phenols. Gallic acid was used as a positive control.

Tannins: For tannins, Gelatin test was followed. To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate confirms presence of tannins in the extract. Tannic acid was used as a positive control.

Flavonoids: Alkaline reagent test was done. To the extract, few drops of sodium hydroxide were added. Intense yellow colour that becomes colourless on adding dilute acid confirms presence of flavonoids. Lemon skin juice was used as a positive control.

Terpenoids: Salkowski test was followed. To 0.5 g of extract, 2ml chloroform is mixed. Then 3 ml of concentrated sulphuric acid was added carefully to form a layer. Reddish brown color formation at the interface confirmed presence of terpenoids in the plant extract.

Protein: Biuret test was conducted in order to test the presence of protein containing CONH group. To about 3 ml of sample, 1 ml of 4% NaOH and few drops of 1 % Copper Sulphate solution are added. A violet or pink colour indicates the presence of protein. Bovine Serum Albumin(Hi Media) @ 1 mg/ml was used as a positive control.

Reducing sugar: Fehling's Test was performed in order to test the presence of reducing sugar in the sample. Fehling A and Fehling B solutions were initially mixed and boiled in 1:1 ratio. Finally, an equal volume of sample solution is added and boiled in water bath for 10 minutes until a red precipitate is obtained indicating presence of reducing sugar. Glucose solution @ 1 mg/ml was used as a positive control.

The experiments were done in duplicates in two independent set ups and similar results were obtained. All the chemicals were obtained from SD Fine Chemicals Limited, India and were freshly prepared.

F. Determination of the antidermatophytic activity

Antidermatophytic activity was carried out using agar incorporation method, as described by Hassan *et al.*, (2007) [16] with some modifications. The fungal isolates (dermatophytes) were cultivated on Sabouraud Dextrose Agar (SDA) medium in petridishes (Borosil, 90 mm diameter). One milliliter of preparation of each extract of the plants were aseptically mixed with 9 ml of liquefied SDA in sterilized Petri Plates (Tarsons, 45mm diameter) and mixed gently until homogeneity. After cooling and solidification of the medium, the seeding was carried out by inoculation of all the dermatophytes isolates in the middle of the petridishes. The treated and control petridishes were incubated at 37°C for 7 days. Three replicates for each concentration were made. Water was used as negative control. Griseofulvin (50mg/ml) was used as a positive control. Presence of growth (+) is a negative test (indicating the non -potency of the drug) and absence of growth (-) is a positive test (showing the potency of the drug).

III. RESULTS AND DISCUSSION

Initially, biochemical profiling of the crude extracts were done. The plant extracts were screened for presence of alkaloid, glycosides, saponins, phenols, tannins, terpenoids, flavonoids, reducing sugar and protein. The results of biochemical tests are presented in Table 2. All the solutions were adjusted to 15mg/ml concentration. The differences in the chemical composition of macerated extracts is very clear. Scores were given on the basis of presence or absence of a



particular trait. The highest score was 8 out of 9, found in four species, *J. adhatoda*, *E. alba*, *C. roseus* and *C. sativa*. This was followed by score 7, for *C. alata* and *A. conyzoides* and *S. calendulacea*, while the lowest score was 5, found in two species, *A. vulgaris* and *P. thyrsiformis*.

All the aqueous extracts were screened using agar incorporation method. The results are given in Table 3. It was found that the extracts from *C. alata*, *C. roseus* and *A. conyzoides* were the most potent. All the three plant extracts showed antidermatophytic activity against the three test fungi. All the organisms exhibited growth in negative control where water was added instead of plant extract. No growth was shown in griseofulvin supplemented media (50 mg/ml).

Silimilarly, Phonhpaichit *et al* (2004) [17] reported antifungal activity of crude methanolic leaf extracts of different *Cassia* species against pathogenic fungi *Microsporum gypseum*, *Trichophyton rubrum* and *Penicillium marneffeii*. The present findings are in agreement with recent work of Ekwealor and Oyeka (2016) [18] who studied the potential of both methanolic and aqueous extract of leaf of *Cassia alata* against common dermatophytes. The antidermatophytic activity of *Catharanthus roseus* was also studied by Gupta *et al* (2014) [19], Kumari and Gupta (2013) [20], Bhadouria and Kumar (2011) [21]. These studies revealed that presence of higher levels of flavonoid, phenol and saponin in the plant extract showed higher antidermatophytic activity. In the present paper, aqueous extracts of *C. roseus* showed good response in concurrence with the presence of bioactive components. On the other hand, Dongmo *et al* (2018) [22] had reported antidermatophytic activities of methanolic extracts of *A. conyzoides*. Therefore, simple aqueous paste can also be applied in case the patient is not responding to any of the treatments. It can be thus concluded that aqueous extracts of the common plants (the so-called 'weeds') can be easily prepared and their potentials can be explored further in order to develop easy formulations for the diseases other than the ringworms.

However, the antifungal activity score could not be correlated with the biochemical component score. This may be due to higher levels of one or the other component.

Contrary to the present findings, 80% of water extracts did not show any antifungal activity [23], which may be attributed to extraction method used and the test fungi. As suggested by Azwanida (2015) [24], further purifications steps of the properly prepared crude extracts is not essential for showing efficacy.

Table 2. Biochemical analysis of crude aqueous leaf extracts.

Plant species	A	G	S	P	T	Te	Fl	R	Pr	Score
<i>J. adhatoda</i>	+	+	+	+	-	+	+	+	+	8

<i>E. scaber</i>	+	+	+	-	-	-	+	+	+	6
<i>S. calendulacea</i>	+	-	+	+	-	+	+	+	+	7
<i>A. vulgaris</i>	+	-	-	+	-	+	+	-	+	5
<i>P. thyrsiformis</i>	+	-	-	-	-	+	+	+	+	5
<i>H. indicum</i>	+	-	+	-	-	+	+	+	+	6
<i>L. zeylanica</i>	+	+	-	-	+	+	+	+	+	7
<i>E. alba</i>	+	+	+	+	-	+	+	+	+	8
<i>C. alata</i>	+	+	-	-	+	+	+	+	+	7
<i>A. paniculata</i>	+	-	-	+	-	+	+	+	+	6
<i>C. roseus</i>	+	+	+	+	-	+	+	+	+	8
<i>A. conyzoides</i>	+	-	+	+	-	+	+	+	+	7
<i>C. sativa</i>	+	+	+	+	-	+	+	+	+	8
Positive control	+	+	+	+	+	+	+	+	+	9
Negative control (double distilled water)	-	-	-	-	-	-	-	-	-	0

A-Alkaloids

G-Glycosides

S- Saponins

P-Phenols

T- Tannins

Te- Terpenoids

Fl- Flavonoids

R- Reducing sugars

Pr-Proteins

‘+’ indicates positive reaction;

‘-’ Indicates absence of positive reaction

Table 3. Determination of antidermatophytic activity using agar incorporation method for aqueous leaf extracts.

Fungal species	<i>T. rubrum</i>	<i>T. rubrum</i>	<i>T. mentagrophytes</i>	<i>T. mentagrophytes</i>	<i>M. audouinii</i>	<i>M. audouinii</i>	Score out of 6
Extract concentration	10 mg/ml	15 mg/ml	10 mg/ml	15 mg/ml	10 mg/ml	15 mg/ml	
Plant species							
<i>J. adhatoda</i>	-	+	-	+	-	+	3



<i>E. scaber</i>	-	+	-	-	-	-	1
<i>S. calendulacea</i>	-	-	-	-	-	+	1
<i>A. vulgaris</i>	-	+	-	-	-	+	2
<i>P. thyrsoformis</i>	-	+	-	+	-	+	3
<i>H. indicum</i>	-	+	-	-	-	-	1
<i>L. zeylanica</i>	+	+	-	+	-	-	3
<i>E. alba</i>	-	+	-	-	-	+	2
<i>C. alata</i>	+	+	-	+	+	+	5
<i>A. paniculata</i>	-	+	-	-	-	+	1
<i>C. roseus</i>	+	+	+	+	-	+	5
<i>A. conyzoides</i>	+	+	-	+	-	+	4
<i>C. sativa</i>	-	+	-	+	-	+	3
Double distilled water	+	+	+	+	+	+	6
Griseofulvin (50mg/ml)	-	-	-	-	-	-	0

(+) Indicates absence of fungal growth (efficacy of extract);
 (-) Indicates presence of fungal growth (inefficacy of extract).

IV. CONCLUSION

The present work highlights the potential of simple aqueous extracts without any laborious extractions of commonly found species of plants for the treatment of dermatophyte-affected patients, which needs further investigation.

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