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Comparative evaluation of extracts of *Betula cylindrostachys* and *Urtica dioica* for antidandruff activity against *Malassezia furfur*

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Context: Betula and Urtica are widely used traditional medicinal plants to treat dandruff. The present article provide scientific basis for the use of these plants in traditional medicine. Aqueous and organic extracts of plants under study were evaluated for anti-dandruff activity and their comparative efficacy with synthetic drug.

Aim: Aim of this study was to evaluate the comparative efficacy of various extracts of selected plants responsible for anti-dandruff activity.

Methods and Materials: Antidandruff activity of successive fractions of crude methanolic extract of *B*. *cylindrostachys* Lindl and *U. dioica* Linn with pet ether (60- 80 $^{\circ}$ C), chloroform, ethyl acetate and n- butanol and total aqueous extract were studied using agar diffusion assay using *Malassezia furfur* (MTCC 1374) strain.

Results: *B. cylindrostachys* chloroform fraction is having good antidandruff activity against *Malassezia furfur* compared to pet ether, ethyl acetate, n- butanol and total aqueous extract. *U. dioica* total aqueous fraction is most active fraction against *Malassezia furfur* compared to organic extracts. Betula chloroform fraction is having comparatively more activity as compared to Urtica total aqueous fraction.

Conclusion: The result shows that the chloroform and aqueous fraction of *B. cylindrostachys* and *U. dioica* respectively has good antidandruff activity compared to other reported extracts and can be used as potential antidandruff substances.

Keyword: Agar diffusion assay, Antidandruff, B. cyindrostachys, Malassezia furfur, U. dioica

Introduction

Dandruff is a common scalp condition resulting due to excessive drying of skin and due to over activity of oil glands known as seborrhea^[1] and seborrhea is the precursor of seborrheic dermatitis^[2]. The severity of dandruff ranges from mild dandruff to exfoliative erythroderma. The treatment options available in market are mostly synthetic one which are having certain limitations like inability to prevent recurrence, compliance issue and poor efficacies etc^[2]. The research into biologically active compounds obtained from natural sources has always been of great interest for scientists looking for new sources of useful drugs.

Materials and Methods Plant material and extraction

Leaves of *Betula cylindrostachys* Lindl. and aerial parts of *Urtica dioica* Linn. were collected in December 2009, from botanical garden of Forest Research Institute (FRI), Dehradoon, India. The collected leaves of *B. cylindrostachys* and aerial parts of *U. dioica* were air dried in shade and the dried samples were then coarsely powdered. The air dried sample was extracted with methanol by hot continuous soxhlation method. The crude methanolic extract was fractionated into various fractions by partioning of 95% aqueous methanolic extract successively with pet ether (60 - 80 °C), chloroform, ethyl acetate and n- butanol to obtain pet ether fraction, chloroform fraction, ethyl acetate fraction and nbutanol fraction³. Total aqueous extracts of *B. cylindrostachys* and *U. dioica* were also prepared. The dried extracts were stored in glass bottles in refrigerator for further use.

Antidandruff Activity

Preparation of test microorganisms

For antidandruff activity evaluation, Malassezia furfur (MTCC 1374- procured from IMTECH, Chandigarh, India) was used. The yeast was maintained on sabouraud- dextrose agar media and incubated at 32 °C for 96 hours. After completion of incubation, the growth on the slant was washed with 10ml sterile normal saline with vortexing. Culture concentration was taken at 560 nm by spectrophotometer. A sterile swab is dipped into the slant containing normal saline and excess inoculums were removed by pressing the swab against the inner wall of the test tube. Inoculums is uniformly spread over the plate and left at room temperature for 20 minutes to dry. Wells were punched in each plate with the help of 6 mm steel agar borer and filled with 0.05 ml of 10mg/ml extract solution. Plates were incubated at 32 °C for 96 hours. Results were noted at the end of incubation period ^[4, 5, 6].

Phytochemical Analysis

The extract having good antidandruff activity was subjected to qualitative chemical screening of active constituents such as carbohydrates, tannins, saponins, glycosides, sterols, flavonoids and alkaloids. The phytocemical screening was performed according to standard procedures ^[7, 8].

Result

The results of our investigation confirmed the antidandruff properties of *B. cylindrostachys* and *U. dioica*. In addition the fractionated methanolic

extract i.e. pet ether, ethyl acetate, choloroform and n- butanol and total aqueous extracts were comparatively evaluated.

Table 1: Zone of inhibition (mm) of different test

 extracts of *B. cylindrostachys* and standard

 ketoconazole

S. No	Extract	Concentration	Zone of Inhibition (mm) Mean± SD
1.	Pet ether	10 mg/ml	9.1 ± 0.36
2.	Chloroform	10 mg/ml	22.47 ± 1.14
3.	Ethyl acetate	10 mg/ml	9.37 ± 0.38
4.	n- Butanol	10 mg/ml	10.86 ± 0.26
5.	Total aqueous	10 mg/ml	11.43 ± 0.42
6.	Ketoconazole (Standard)	10 µg/ml	25 ± 0.0

The above mentioned readings are inclusive of disc diameter.

Values are expressed as mean \pm standard deviation, where n = 3.

Table 2: Zone of inhibition (mm) of different test

 extracts of U. *dioica* and standard ketoconazole

S. No	Extract	Concentration	Zone of Inhibition (mm) Mean± SD
1.	Pet ether	10 mg/ml	-
2.	Chloroform	10 mg/ml	12.13 ± 0.35
3.	Ethyl acetate	10 mg/ml	7.53 ± 0.15
4.	n- Butanol	10 mg/ml	12.0 ± 0.26
5.	Total aqueous	10 mg/ml	16.47 ± 0.65
6.	Ketoconazole (Standard)	10 µg/ml	25 ± 0.0
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The above mentioned readings are inclusive of disc diameter.

Values are expressed as mean \pm standard deviation, where n = 3.

The pet ether and ethyl acetate fraction of Betula have shown lesser activity against *Malassezia furfur* species as compared to moderate activity of n- butanol and total aqueous fraction. Good activity was observed with chloroform extract of Betula.

The Total aqueous extract of Urtica had shown good antidandruff activity as compared to organic

extracts of urtica against the yeast Malassezia furfur.

The total aqueous extracts of Urtica and chloroform extract of Betula has shown to be more active with larger Zone of Inhibition and shown good promising antidandruff activity.

The phytochemical screening results indicated the presence of phenolic compounds, steroids and terpenoids in Betula chloroform extract whereas significant flavonoid content were present in Urtica aqueous extract in addition to steroids and glycosides.

Antimicrobial mode of action may be due to the ability to inactivate microbial adhesions, cell envelope transport proteins, enzymes or their ability to combine with polysaccharides. Antimicrobial mode of action seems to involve membranolytic properties ⁹ for effective results.

Discussion

The commercially available synthetic antidandruff drugs (Ketoconazole) showed a larger inhibitory effect as compared to natural extracts. This may be attributed to the fact that, as plant extracts are preceived as more dilute because of crude form compared to purified synthetic agents.

The current synthetic treatment options available have limitations of poor efficacy, recurrence and compliance issue. The herbal drugs may prove to be an alternative for these shortcomings. The chloroform extract of *B. cylindrostachys* and aqueous extract of *U. dioica* having good antidandruff activity; can be further explored for lead molecules responsible for the activity and to extend further research.

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