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In-vitro antifungal activity of various solvent extracts of *Elytraria acaulis*

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Abstract
Aim: The aim of the present study was to analyse the antifungal activity of the various solvent extracts of *Elytraria acaulis* Lindau from the family of Acanthaceae.
Methods: Various solvent extracts were prepared using Soxhlet apparatus. Antifungal activity of *E. acaulis* of various solvent extracts was tested by disc diffusion method, Minimal Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of the extracts was determined by macro broth dilution assay.
Result: The methanol extracts of *Elytraria acaulis* at100µg showed maximum antifungal activity against *C. albicans* followed *by C. Krusei.* Fluconazole was used as positive control. The acetone and chloroform extracts showed moderate and low antifungal activity against all the testes pathogens. The MIC values of the extracts were ranged between 1.25 and 160 µg mL^{-1}. The MFC values of the extracts ranged between 1.25 µg mL^{-1} and 10 µg mL^{-1}.

Keywords: Antifungal, MIC, MFC and fluconazole

1. Introduction
Fungi are eukaryotic and many fungicides exhibits pollution which infects all the other eukaryotes and exhibit lower potency under filed conditions, cause chemical pollution, poisoning fruits and vegetables essential in human diet. *Aspergillus* species are toxigenic, contaminate food and produce mycotoxins. Fungal pathogens are a major problem in agriculture, as most of the fungicides employed exhibit lower potency under field conditions and have been a source of chemical pollution, poisoning fruits and vegetables, which form an essential component of the human diet [1].
Many species of yeasts are harmless, unless *Candida* is the major pathogen involved in fungal infections worldwide. *C. albicans* are most important species and it is responsible for oral thrush, candidiasis, candiduria and Candidemia frequently seen in patients and it is also responsible to cause vulvovaginitis in girls at the pubeteric age group. The incidence of *Candida* species is significantly increases over the past two decades and non-albicans *Candida* (NAC) continue to replace *C. albicans* at most of the clinical sites i.e. blood stream infections particularly higher in vagina during pregnancy [2].
Medicinal plants are a rich source of antimicrobial compounds and many of the pharmaceuticals used in traditional medicine are readily available in rural areas at relatively cheaper than modern medication [3]. Plants generally consist of many secondary metabolites which are important source of microbicides, pesticides and many pharmaceutical drugs. Plant products still remain the principal basis of pharmaceutical agents used in traditional and modern medicine [4]. Aim of the present study was to analyse the antifungal activity of various solvent extracts of *Elytraria acaulis* crude extracts.

2. Materials and methods
2.1 Preparation of plant extracts
The collected Plant material was washed cleanly in tap water and then air dried under shadow condition at room temperature (25 ºC) for 2-3 weeks until they become brittle. After complete drying, the plant material was ground to fine powder using electrical blender. Fifty grams of dried powder was packed in the Soxhlet apparatus with 300 ml of solvents (methanol, acetone, Chloroform and hexane) extracted until the extract was clear.

The solvents from the extracts were evaporated using a rotary vacuum evaporator and the extract was stored in a refrigerator for further use. Solvents from the extracts were evaporated using a rotary vacuum evaporator and the extract was stored in a refrigerator for further use.

2.2 Fungal culture
Five different fungal cultures were procured and used in our present study. *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Candida albicans* and *Candida krusei*.

2.3 Antifungal assay
Sabouraud's dextrose agar plates were prepared and inoculated with the fungal cultures. The *Elytraria acaulis* crude solution was prepared with and dissolved in 5% DMSO. In sterile empty discs were loaded with the prepared solution and allowed to dry. Fluconazole was used as positive control and DMSO as negative control.

2.4 Minimum inhibitory concentration
The minimum inhibitory concentration of the crude extracts off E. acaulis plant was tested at various concentrations.
The plant extract was dissolved in DMSO to obtain 0.62, 1.25, 2.5, 5, 10, 20, 40, 80, 160 and 320. Standardized suspension of 50 µl of the test organism was inoculated and incubated at 28 °C for 2 days (yeasts) and 3 days (moulds). The lowest concentration without any growth was determined as the minimal inhibitory concentration and Minimum bactericidal concentration was performed for those MIC.

2.5 Minimum fungicidal concentration
Minimum fungicidal concentration was determined was determined by subculturing the wells showing no growth in Saboraud's Dextrose agar plates. Plates were incubated and the plate without growth obtained from the lowest extract concentration was selected as the MFC value.

2.6 Activity Index
Following formula was used to measure Activity Index,

$$\text{Activity Index} = \frac{\text{Zone of inhibition of extract}}{\text{Zone of inhibition of antibiotic}}$$

Zone of inhibition of stocks at different temperature was measured and similarly zone of inhibition of Antibiotic was measured.

3. Results
Antifungal potential of the Crude extracts were examined and the results were presented in the table 1. All the extracts showed activity against the tested fungal pathogens in which the methanol extracts showed maximum antifungal activity against the yeasts *C. albicans* (14±0.4 µg/ml) and *C. krusei* (13±0.57 µg/ml) followed by the mold *Aspergillus fumigatus* (11±0.3 µg/ml) and *A. niger* (11±0.22 µg/ml) species.
The MIC of the crude extracts was screened and the results were presented in the Figure 1. In which all the crude extracts showed minimum inhibitory concentrations against the tested pathogens ranging from 20 to 160 µg/ml concentrations. The methanol sample showed lower MIC of <20 µg/ml concentrations against *A. flavus, A. fumigatus, C. albicans* and *C. krusei* and <40 µg/ml against *A. niger*. The activity index related towards positive control concordance with the crude extracts showed good antifungal activity.

4. Discussion
In last few decades, there has been a gradual reinforcement of interest in the application of medicinal plants in developed, as well as in developing countries and the medicinal properties of several plants have been explored. In search for medicinal property of any plant basically depends on the solvent utilized in the process of extraction [5].
Antifungal agents presently available in the market are inadequate due to their toxicity, low effectiveness, and cost for drawn out treatment. Therefore, there is a need to increase antifungal compounds potency which can satisfy the current circumstances. The efficacies of the extracts were also compared with the activity of the standard antibiotics available.
The results of present the study showed the antifungal efficacy of all the extracts were more promising when compared with the standard antibiotics. Significant differences were observed when comparing the un-purified crude extracts with the purified drugs. The agar-cup diffusion method is commonly employed for preliminary susceptibility testing, but it is not a reliable and perfect method for assessment, because of the high degree of interference due to inconvenience in drug diffusion [6].
Yucesoy *et al.* [7] reported an important factor relating to the susceptibility of fungal cultures while growing the *Candida* species in blood agar and SDA agar. The total inhibition rates obtained by using blood agar suggested that *C. albicans* and *C. tropicalis* were the most inhibitive strains. *C. krusei* was found to be the least inhibitive strain. Our results are also in coincidence with them as it was cultivated early in blood agar and later the antimicrobial activity was tested in SDA agar. *C. albicans* is the most sensitive strain and no significant difference was detected on blood agar and SDA results. In our present study methanol extracts showed good significant antifungal and MIC activities. Similarly in a previous study it was reported that methanolic extract Aconitum plants showed significant antifungal activity [8]. Researchers reported that the methanolic extracts of the *E. acaulis* plant showed the presence of many secondary metabolites compared to other solvents. The different parts of the plant extracts showed broad spectrum of antimicrobial activity against some human pathogens [9]. Due to the high polarity of methanol majority of the secondary metabolites were extracted and the antimicrobial activity was high.

5. Conclusion
From the present investigation, it is concluded that the methanol extract of *E. acaulis* has significant *in vitro* antifungal activity against the urinary tract infection causing *Candida* and food poison inducing *Aspergillus* species.

Table 1: Antifungal activity of *E. acaulis* extracts

Fungal pathogens	Zone of inhibition of Various solvent extracts of *E. acaulis* (100 µg concentration)								
	Hexane	AI	Acetone	AI	Chloroform	AI	Methanol	AI	Positive
A. niger	NA	0	07±0.42	0.47	09±0.39	0.60	11±0.22	0.73	15±0.3
A. flavus	NA	0	07±0.20	0.47	08±0.45	0.53	10±0.47	0.67	15±0.55
A. fumigatus	NA	0	08±0.32	0.50	09±0.56	0.56	11±0.3	0.69	16±0.28
C. albicans	09±0.27	0.47	11±0.4	0.57	12±0.25	0.63	14±0.4	0.74	19±0.43
C. krusei	07±0.32	0.44	09±0.47	0.56	11±0.59	0.69	13±0.57	0.81	16±0.27

Positive control- Fluconazole (25 µg); AI – Activity Index; Values are represented in Mean ±S.D. NA- no activity

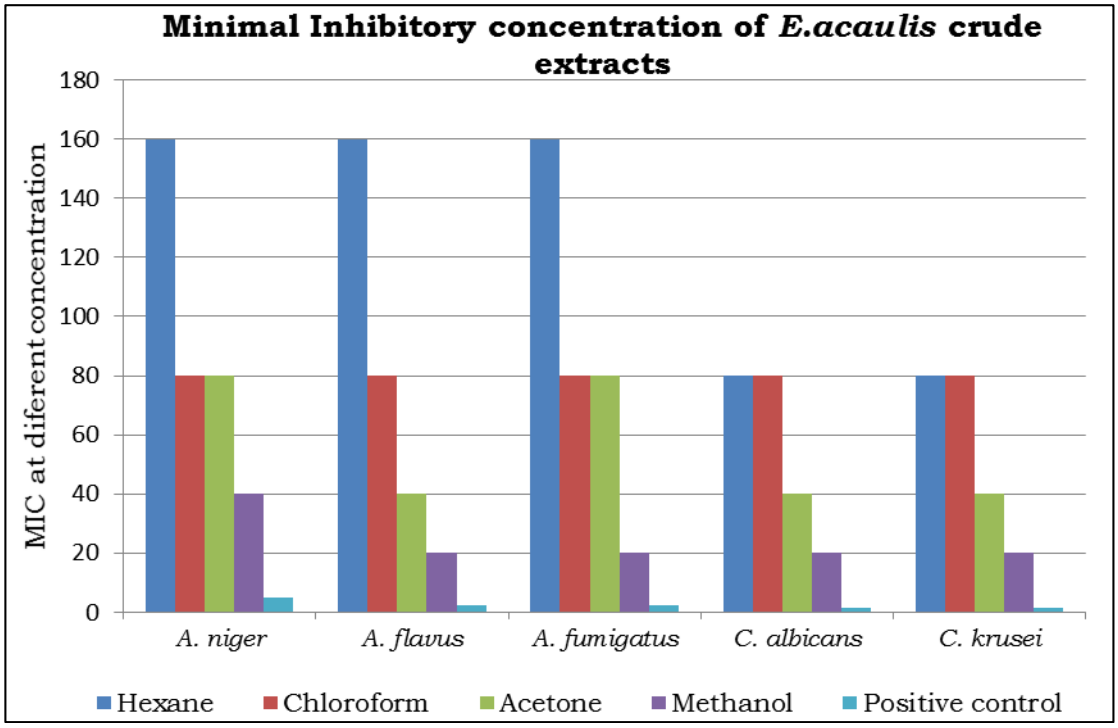

Fig 1: Minimal inhibitory concentration of *E. acaulis*

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