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Antifungal Activity of Glycyrrhiza *glabra* Linn. and *Emblica Officinalis Gaertn*. by Direct Bioautography Method

Tharkar P.R.[1]*,Tatiya A.U.[2], Shinde P.R .[2], Surana S.J.[2], Patil U.K.[3]

[1]S.G.S.P.S. Institute of Pharmacy, Kaulkhed, Akola,MH,India

[2]Department Pharmacognosy, R.C. Patel Institute of Pharmaceutical Education and Research Shirpur. Dist Dhule, ,India

[3]Department of Microbiology and Biotechnology, H. R. Patel College of Arts, Science and Commerce, Shirpur, Dist Dhule, Maharashtra,India

Corres. author: priyankatharkar@rediffmail.com
Mobile no: 09922540733

Abstract: Direct bioautography is a method to localize antibacterial activity on a chromatogram. Optimized direct bioautography is useful both for the analytical determination of the main compounds and for characterization of their antibacterial effects. In the present study, the hydro alcoholic extract of *Emblica officinalis* L. and *Glycyrrhiza glabra* was investigated for antifungal activity against *Candida albicans* (*C. albicans*) and *Aspergillus niger (A. niger*) conventionally and by direct bioautography. Zone of inhibition for *G. glabra* were 23.83 mm and *E. officinalis* 18.10 mm in diameter at conc.1mg/ml against *C. albicans* while zone of inhibition produced by *G. glabra* and *E. officinalis* were 26.41 mm and 10.28mm in diameter at 2 mg/ml respectively against *A. niger*. MIC range of 512-1024 µg/ml and 1024-2048 µg/ml for *C. albicans*. While 256-512 µg/ml and 1024-2048 µg/ml For *A. niger* respectively. In TLC bioautographic studies it shows the significant inhibitory effect against *A. niger*.
Keywords: Antifungal activity, Bioautography, MIC, *Glycyrrhiza glabra* Linn, *Embellica Officinalis*.

Introduction

Plants have supplied over 25% of prescription drugs used in human medicine and such pharmacologically active plants have also provided leads to natural pesticides. India has an extraordinarily rich flora and wide knowledge of indigenous medicinal plants is well documented. Accordingly, we are investigating the potential of indigenous medicinal plants as a resource for new biofungicides.

G. glabra is dried underground stems and roots (*Leguminoceae)*. It is widely cultivated in Punjab and sub Himalaya tracts. Traditionally it is used as antiallergic, demulcent, emollient, fungicide, peptic ulcer [1,2]. *G. glabra* showed the presence of flavonoid, saponin, triterpenoid, sterols, resins and glycosides [3,4]. *E. officinalis* is a dried ripe fruits *(Euphoebiaceae)*. It grows throughout tropical and subtropical India. It is traditionally used as astringent, carminative, digestive, diuretics, bronchitis, grayness of hairs [5,6]. *E. officinalis* showed the presence of flavonoids, saponin, triterpenoids, sterols, glycosides, polyphenols and tannins [7]. Antibacterial activity of compound obtained from *G. glabra* and *E. officinalis* were reported. In the present communication antifungal activity of hydro alcoholic extract of *G. glabra* and *E. officinalis is* reported by bioautography method.

Materials and Methods

The roots of *G. glabra* and the fruits of *E. officinalis* were collected from local market of Shirpur, (Dhule district). Plant materials were authenticated by Dr. D. A. Patil, Plant Taxonomist, S.S.V.P.S. Science College Dhule (MS).

Preparation of the Extract:

All the drugs were powdered and macerated with hydro alcoholic solvent (70:30), with intermediate shaking on the mechanical shaker and kept to macerate (cold maceration) for 3 days, the extracted matter was separated by filtration and then concentrated by rotary vacuum evaporator. After complete drying preserved in the desiccator for further studies.

Microorganisms:

The test organism includes: *Aspergillus niger* (NCIM 3471) and *Candida albicans* (NCIM 545). Fungal strains were obtained from Department of Microbiology and Biotechnology, H. R. Patel College of Arts, Science and Commerce, Shirpur, Dist. Dhule, Maharashtra (India).

Evaluation of Antifungal activity:

Antifungal activity by disk diffusion method [8], MIC by double dilution method [9] and by direct bioautography method.

Antimicrobial assay:

The inoculums of test organisms were sprayed on PDA plates. Wells of 6 mm were punched into the agar medium and filled with 0.1 ml of extract solution having concentration 1000µg/ ml, compared with control the plates were incubated for 24 hours at 37°C. The antifungal activity was evaluated by measuring the zone of inhibition against test organism.

Direct bioautography method [10]:

Mobile phase used for *G. glabra*: $CHCl_3$: MeOH: GAA: H_2O (2:0.5:0.8:0.2) and for *E officinalis*: Ethanol 10% acetic acid. 20 µl of solutions corresponding to 1000 µg of crude extracts were applied on Si gel TLC plates, developed with respective mobile phase and dried for complete removal of solvents. Aliquots of 25-50 ml of inoculums spray solution (ca. 3 x 105 conidia/ml) were prepared for *A. niger* with liquid potato dextrose (potato 200 g, dextrose 20 g and water to make total volume of 1 L.). Using a 100 ml chromatographic sprayer, plates were sprayed with the spore suspension of *A. niger* lightly (to a damp appearance) three times and incubated for four days in darkness in a moist chamber at 25°C. The experiment was repeated twice and similar results were obtained.

Results and Discussion

After developing TLC plates in the respective solvent system and observing them under UV cabinet at 365 nm , six different spots were observed for *G. glabra* having R_f values 0.14, 0.32, 0.39, 0.47, 0.75 and 0.83 and for *E .officinalis* two spots were observed having R_f values 0.41, 0.79. In case of *G. glabra* out of the six spots the microbial growth was observed on one spot having R_f 0.39 While in case of *E.officinalis* there was no growth on the track of solvent, but growth was observed at the periphery.

The hydro alcoholic extract of the *G. glabra* and *E. officinalis* showed the significant antifungal activity than *E. officinalis*. Selection of the plants from the literature were made on the basis of their common use in the treatment of skin diseases, given in Ayurvedic as well as modern text books, so one can say the claims made by traditional system of medicine are true and the claims proved in this research with the scientific evidence.

Table 1. Results for Antifungal Activity:

			Zone of Inhibition (mm)		MIC (µg/ml)	
Sr. no.	**Drug**	**Conc. (mg/ml)**	***C. albicans***	***A. niger***	**C. *albicans***	***A.niger***
1.	*G. glabra*	1	23.83	26.41	512- 1024	256-512
2.	*E. officinalis*	2	18.1 0	10.28	1024-2048	512-1024

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