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ANTIFUNGAL ACTIVITY OF *EMBLICA OFFICINALIS* SEED EXTRACT

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ABSTRACT

The present study was undertaken to know the antifungal activity of *Embllica officinalis* seed by In-Vitro evaluation. Collected plants were extracted by using methanol in soxhlet apparatus. Pathogenic organisms namely *Malassezia furfur* was tested with antibacterial activity of crude samples in different solvents by using Disc diffusion technique was compared with standard drug (ketoconazole) petriplates were incubated at 37°C for 48 hours the zone of inhibition is measured. The TLC was carried out to know seed extract of *E. Officinalis* is to be standard.

Keyword: Evaluation, Antifungal Activity, Malassezia furfur, Zone of inhibition, Antidandruff

INTRODUCTION

Seborrheic Dermatitis (SD) and dandruff are of a continuous spectrum of the same disease that affects the seborrheic areas of the body [1]. Dandruff is restricted to the scalp, and involves itchy, flaking skin without visible inflammation. SD can affect the scalp as well as other seborrheic areas, and involves itchy and flaking or scaling skin, inflammation and pruritus [2]. Various intrinsic and

environmental factors, such as sebaceous secretions, skin surface fungal colonization, individual susceptibility, and interactions between these factors, all contribute to the pathogenesis of SD [3, 4]. Treatment of SD and dandruff focuses on clearing signs of the disease; ameliorating associated symptoms, especially pruritus; and maintaining remission with long-term therapy. Because

the main underlying pathogenic mechanisms involve *Malassezia* proliferation and local skin irritation and inflammation, the most common treatment is topical antifungal and anti-inflammatory [5-8]. Although modern medicine and pharmaceuticals have now largely replaced traditional medicine as the mainstream treatment for human disease, herbalism is still widely practiced around the world [9]. The selection of plants as the primary source of medicine by multiple cultures over the past millennia was no accident. The chemical space contained within the plant kingdom is astronomical, providing the probabilistic basis for hitting the right mechanistic targets underlying various maladies [10, 11]. Moreover, a myriad of plant specialized metabolites evolved to mediate interspecies chemical communications, and therefore were adapted to possess drug-like properties. When consumed by humans, these compounds are well placed to interact with human protein targets, or to alter the growth of commensal, pathogenic or parasitic organisms living inside the human body, which in turn impact human health and disease states [12, 13].

MATERIALS AND METHODOLOGY

Plant Collection

Plants were collected with the help of manual like clippers, diggers, scrapers, etc by the lab

technicians of Department of Pharmacognosy, College of Pharmacy, Chopda (Jalgaon, Maharashtra) and were collected in plastic bags. Further, the plant and its constituents were authenticated by Botanical Survey of India, Pune.

Experimental pathogenic organisms

In-vitro study was conducted on *Malassezia furfur* culture. The Parasites were maintained under standard laboratory conditions at 37 ± 0.5 .

METHODOLOGY FOR THE STUDY

Extraction of Active Constituents from *Emblica Officinalis* Seed [14]

Collected plant parts were air dried under shade and then ground to a coarse powder using a grinder. Powdered seed material was extracted first with petroleum ether for defatting and then extracted with methanol in soxhlet apparatus. Further, the methanolic extract of amla seed powder was polarity based fractionated using a separating funnel by chloroform. Extracts and fractions were filtered and the solvent was evaporated to dryness under natural conditions. The final extract was stored in well closed containers under refrigeration (2- 4°C) until testing.

TLC Development and Standardization of *E. Officinalis* Seed

Amla Powder Extract was weighed 500mg of Amla seed powder was extracted with 50ml of methanol by heating under reflux

condition. The extract was filtered by Whatmann filter paper and used for TLC. Mobile Phase used was Ethyl Acetate: Acetic Acid Glacial: Formic Acid: Water (6:1:1:2 v/v/v/v) Amla Powder 10mg/ml [10 μ l] Stock solution was prepared and was spotted on precoated silical plates and was air dried. Further the plate was introduced in a saturated chamber and was runned by mobile phase for its 80 percent height and was observed under a UV chamber. The TLC was carried out in comparison with Ascorbic acid stock solution. The Rf values were calculated and were found to be in line with the standard. Thus by this process, the seed extract of *E. Officinalis* was established to be standard.

In vitro pharmacological evaluation of *Emblica Officinalis* seed extract for its Antifungal activity on *Malassezia furfur* culture

The crude extract of seed was tested for antidandruff antifungal activity. Drug ketoconazole (10 μ g) and DMSO used as control. Disc diffusion technique was employed to test the antibacterial activity of crude samples in different solvents against pathogenic organisms namely *Malassezia furfur*. The nutrient agar plates were inoculated with 0.1 ml of pathogenic microbes by spread plate method. The

whatmann filter paper disc were sterilized and inoculated with the samples and DMSO was kept as negative control. All the plates were incubated at 30°C for 24 hours to measure the zone of inhibition. The petriplates were incubated at 37°C for 48 hours the zone of inhibition was measured.

Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration was determined by serial dilution method. Serial dilution of various concentration extract was individually placed in plates. The lowest concentration of each extract in various solvents showing zero growth of bacteria after 24 hours were recorded as MIC [12].

Relative Percentage of Inhibition

The relative percentage of inhibition of seed extract was compared with positive control was calculated by $100x((x-y)/(z-y))$ where x =total area of inhibition of test extract; y = total area of inhibition of solvent; z = total area of inhibition of standard drug [11].

RESULT & DISCUSSION

TLC estimated comparison of *Emblica Officinalis* Seed (Figure 1).

Disc diffusion technique was employed to test the antibacterial activity of crude samples in different solvents against pathogenic organisms namely *Malassezia furfur*. The activity of the seed powder

extract fraction was compared with standard drug (ketoconazole). The nutrient agar plates were inoculated with 0.1 ml of pathogenic microbes by spread plate method. The whatmann filter paper disc were sterilized and inoculated with the samples and DMSO was kept as negative control. All the plates were incubated at 30°C for 24 hours to measure the zone of inhibition. The petriplates were incubated at 37°C for 48 hours the zone of inhibition was measured for MIC and Relative percentage of inhibition (Table 1).

Relative Percentage of Inhibition

The relative percentage of inhibition of seed extract was compared with positive control was calculated by $100 * [(x-y)/(z-y)]$ where x =total area of inhibition of test extract; y = total area of inhibition of solvent; z = total area of inhibition of standard drug. Therefore,

Relative percentage of inhibition of *E. Officinalis* seed extract = $100 * [(19.7-19)/(19.9-19)]$ Relative percentage of inhibition of *E. Officinalis* seed extract = 77 %.

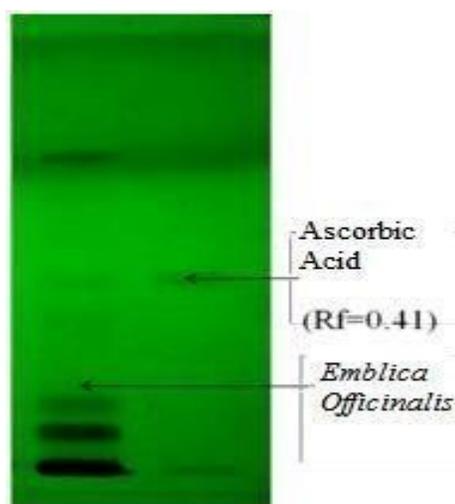


Figure 1: TLC estimation of Ascorbic acid in comparison with *E. Officinalis* seed extract Minimum Inhibitory Concentration (MIC)

Table 1: MIC Values

Test organism Culture	Standard Drug value (10mcg) Zone of inhibition (mm)	<i>E. Officinalis</i> seed extract antifungal activity value Zone of inhibition (mm)
Malassezia Furfur	19.9	19.7

CONFLICT OF INTEREST

Authors have no conflicts of interest to declare.

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