

## **Antifungal Evaluation of Alcoholic Extract of *Euphorbia zeylanicum***

**Somkant V. Jawarkar\*, Sandeep R. Kane<sup>1</sup>**

**\*S. D. Patil Institute of Pharmacy, Islampur Tal-Walwa Dist-Sangli, India**

**<sup>1</sup>Rajarambap College of Pharmacy, Kasegaon Tal-Walwa Dist-Sangli, India**

**Abstract :** This study was to evaluate extracts of *Euphorbia zeylanicum* for anti-fungal activity, cactus-like plant of the family *Euphorbiaceae* commonly called shend by the people in sangli district. The stem-bark and latex of this plant were extracted using 50%-methanol, water and absolute methanol. Antifungal effect of the extracts was evaluated using the Time kill assay. Strains of *Aspegillus*, *Rhizopus*, *Mucor*, *Rodotorula*, *Geotricum*, *Basidiobolus*, *Trichophyton*, *Microsporum*, *Epidermophyton* and *Candida* species were used as test fungi for the study. The extraction of the stem-bark yield 18%, 15% and 25% for absolute methanol, water and 50% methanol, respectively, while the latex yield 13%, 12% and 15% for absolute methanol water and 50% methanol extracts respectively. There was a significant difference in the growth inhibition by the 50% methanol extracts of the stem-bark and latex ( $P=0.5$ ) with significant means of 5.361 and 7.1086 respectively. *Candida albicans* was the most susceptible of the yeasts tested ( $MIC_{90}$  0.313 mg/ml) and significant mean of, 0.896 and *M. gypseum* the least susceptible of the dermatophytes tested significant mean 14.641. In the time kill assay, the results showed that *T. mentagrophytes*, *M. gypseum* and *E. floccosum* cells were killed by the higher concentrations (4 MIC and 2 MIC) of the plant extracts. The plant extracts showed broad spectrum of activity against fungi tested.

**Keywords :** Evaluation, antifungal, time kill assay, *Euphorbia zeylanicum* extracts.

### **Introduction:**

The diseases caused by multidrug resistant fungal pathogens (e. g dermatophytes like *Trichophyton*, *Microsporum* and *Epidermophyton* as well as opportunistic fungi like *Aspergillus species*, *Candida albicans*, *Mucor* and *Rhizopus* species) are becoming the world's leading cause of health complications if not death, especially in immunosuppressed patients<sup>1</sup>. Though fungal diseases (especially superficial and systemic) appear to be rare, they are not easily treated when contacted and thus most often, some cases of HIV AIDs, Hepatitis B, Cancer etc. and other immunosuppressive diseases become complicated by some of these fungal infections<sup>2</sup>. The treatment of immunocompromised, AIDS and cancer patients becomes sometimes difficult due to this problem<sup>3</sup>. This is even worst in under developed countries like Nigeria, where healthcare facilities are not easily

assessable by the vast majority of the population. Greater parts of the population in the interior areas of these countries therefore, rely on plant sources for treatment of skin diseases and other mycotic infections. The investigation of these plants used in folklore medicine for skin infections could invariably be a source of the antimycotic agents urgently needed in less developed world today. Some plants of the genus *Euphorbia*, have been used in folklore medicine from creation till today e. g *Euphorbia kamerunica* and other plants belonging to the family *Euphorbiaceae* have been used to treat skin infections such as ulcers, warts, cancers, tumors<sup>4,2</sup>. *E. tirucalli* has been used for treatment of swelling, asthma, cough, skin problems and rheumatism. Also its latex is used as treatment for sexual impotence, warts, toothache, hemorrhoids, and snake bites. *Euphorbia granulate* has also shown inhibitory activity against Human immunodeficiency virus (HIV-1) protease<sup>5</sup>. This study therefore seeks to explore the largely unexplored rich natural constituents of *Euphorbia zeylanicum*, with the aim of evaluating the antimycotic activity. This plant *Euphorbia zeylanicum* is an erect, eight-angled branched tree with deep angles edged with a border of closely packed paired spines (about 1cm long)<sup>6</sup>.

## Materials and Methods

### Collection of Plant Materials

The plant parts used for this study were *Euphorbia zeylanicum* stem bark and the latex. These were collected from south West region of sangli District. In the Department of Botany, Sangli District the plant was authenticated and the voucher specimen also deposited at Dept of Botany Y.C. College Karad. The latex or sap of *Euphorbia zeylanicum* was collected by cutting open, parts of the bark on the stem and branches. A container was connected to the bottom of the opening from which the latex dripped into the container. It was then allowed to dry in the water bath at 56°C and stored in a close capped bottle pending its use. The plant stem-bark was rinsed thoroughly in running tap water, cut into tiny pieces and air dried in the dark. The dried material was then ground to powder in a mortar, weighed and stored in plastic bags in the dark.

### Extraction of plant materials

Approximately 400 ml of solvent (absolute methanol, water or 50% methanol in water) in a 1 L conical flask was used to soak a 100 g weight of powdered plant material and then covered with cotton wool plugs. After vigorous shaking, the flask was intermittently shaken for 24 hours leaving it in a water bath maintained at 40°C between the intervals of shaking. Three layers of clean muslin cloth were first of all used to filter the mixture before passing it through Whatman no 1 filter-paper. The filtrates were evaporated to dryness in a water bath at 56°C and the percentage yields of the crude extract determined<sup>7</sup>.

### Test Organisms

Trichophyton mentagrophytes, Trichophyton tonsurans, Trichophyton violaceum, Microsporum gypseum, Microsporum canis, Epidermophyton floccosum, Candida albicans, species of Aspergillus, Basidiobolus, Rhizopus, Mucor, Rhizopus and Geotrichum, all collected from the Microbiology laboratory of the Y. C. College Karad, were the fungi used for the study. They were cultured on Sabouraud Dextrose Agar plates at 25-35°C for 48 hours or more and the resultant pure mature colonies were sub-cultured on Sabouraud Dextrose Agar slants and stored as stock cultures.

Fungal stock cultures were sub cultured on Sabouraud Dextrose Agar and incubated at 25-35°C for 7 to 14 days. The matured fungal growths were covered with 2ml of distilled water and gently probed with a sterile loop or the tip of a Pasteur pipette. The resulting suspensions were transferred to sterile test tubes and allowed to settle for about 3-5 minutes. The resultant supernatant suspensions were drained into sterile bottles. The spores or yeast cells (colony forming units (CFU)) in the suspensions were counted using a haemocytometer and the suspensions were then diluted with Sabouraud dextrose broth to correspond to the final standard inoculate suspension (spores or yeast cells) of approximately  $1 \times 10^5$  colony forming unit per ml<sup>8</sup>.

### Determination of the Effects of Plant Extracts on Viable Colony Forming Units (CFU) of the Fungi Using Time-Kill (Inhibition) Assay<sup>7</sup>

The effects of 50% methanol extracts of *Euphorbia zeylanicum* were evaluated by a time-kill assay using the macrobroth dilution technique. The extracts were reconstituted in 20% Dimethyl Sulfoxide (DMSO) and appropriately diluted to the required concentrations. The inoculum size was determined

according to the type of fungus, (e.g.  $1 \times 10^6$  for *Candida albicans*; and  $1 \times 10^5$  for dermatophytes). About 1.00ml of the extract was added to 9 ml of Sabouraud dextrose broth, seeded with the appropriate concentrations of the test fungus to achieve concentrations equivalent to 0.5 x MIC, 1 x MIC, 2 x MIC, or 4 x MICs values. Two sets of control tubes were included for each experiment. One set was seeded with the organism in broth without extract, and the other set contained broth without organism and extract. The control drug Miconazole was similarly diluted. All the fungal cultures were incubated at 25-35°C for  $\geq 48$  hours. Immediately after inoculation of the tubes, aliquots of 100  $\mu$ l of the negative control tubes contents were taken, serially diluted in saline and seeded on nutrient agar plates to determine the zero hour counts. The same was done for the tubes which contained the test fungi after 0 hour, 3 hours, 6 hours, 24 hours and 48 hours respectively. After incubation, the emergent colonies were counted and the mean count (CFU) of each test organism was determined and expressed as  $\log_{10}$ . The Minimum Lethal Concentrations (MLCs) of the extract were the lowest concentrations that gave 99.9% to 100% killing <sup>7</sup>.

## Results

### Antifungal activities of *Euphorbia zeylanicum* Extracts

The results of the antifungal activities of 100mg of *E. zeylanicum* stem- bark showed that the extract inhibited the growth of all the fungi tested. However, the 50% methanol extract showed better activity (14days or more) than the absolute methanol and aqueous extract (12 days or more) (Table 1). *Euphorbia zeylanicum* latex extracts, also showed similar activity on the fungi tested like the stem bark but inhibition of growth was for lesser number of days. The 50% methanol extract inhibited the growth of all the dermatophytes for 12 days or more (Table 1). The control anti mycotic agent (Miconazoleazole) inhibited the growth of all the fungal isolates tested for over 14days, including *Aspergillus*, *Mucor*, *Rhizopus* and *Geotricum species* (Table 1).

**Table 1. Periods of inhibition (in days) of the growth of some pathogenic fungi by plant extract**

Stem-bark	<i>B. h</i>	<i>R. s</i>	<i>C. a</i>	<i>T. m</i>	<i>T. r</i>	<i>T. t</i>	<i>T. v</i>	<i>M. g</i>	<i>M. c</i>	<i>E. f</i>	<i>A. fl</i>	<i>A. fu</i>	<i>M. s</i>
A. methanol	>12	>12	>12	>12	>12	>12	>12	>12	>12	>14	>14	>12	>12
Aqueous	12	12	12	12	12	12	12	12	12	>12	12	12	12
50% Methanol	>14	>14	>14	>14	>14	>14	>14	>14	>14	>14	>12	>12	>12
Latex													
A. methanol	12	12	>12	>12	12	12	12	12	12	>12	12	12	12
Aqueous	12	12	12	12	12	12	12	12	12	12	12	12	12
50% Methanol	>14	>14	>14	>14	14	14	14	>14	14	>14	14	12	12
Miconazole(16 $\mu$ g/ml)	>14	>14	>14	>14	>14	>14	>14	>14	>14	>14	>14	>14	>14

B. h=Basidiobolushapto sporus, R. s= Rhodotorula species, C.a= Candida albicans, T. m= Trichophyton mentagrophytes, T.r=Trichophyton rubrum, T.t=T. T.v=Trichophyton violaceum, M.g= Microsporum gypseum, M.c= Microsporum canis, E.f= Epidermophyton floccosum, A.fl.= Aspergillus flavus, A.fu=Aspergillus fumigatus, G.S=Geotricum species, R.n=Rhizopusnigricans, >=more than, A. n= Aspergillus niger. A.methanol=Absolute methanol

## Discussion

The study of antifungal activity of crude extracts (absolute methanol, water and 50% methanol) of *Euphorbia zeylanicum* by agar dilution and macrobroth dilution method showed that the extracts inhibited the growth of the test organisms. In the agar dilution, there was no significant difference between the activity of the stem-bark and that of the latex extracts. Also there was no significant difference between the activity of the absolute methanol and the aqueous extracts even though the methanol extract were more active when compared with the aqueous extract. The slight difference in the activity might have been probably due to the solubility of the chemical and bioactive constituents in the extracting solvents (methanol and water). The antifungal activity of methanol and aqueous extracts of other *Euphorbia* species have also been reported by other researchers <sup>9-14</sup>. The 50% methanol extracts of the two plant parts (stem-bark and latex) showed significantly higher antifungal activity than the absolute methanol and aqueous plant extracts. In the macrobroth dilution method the results showed that at a significant value of  $\alpha=0.01$  the 50% methanol extract of the stem-bark was more active than

the 50% methanol extract of the latex. They were significant at mean values of 5.361 for stem-bark extract and 7.109 for the latex extract. This activity was dependent as greater inhibition of growth was observed at high concentrations than at lower concentrations in the Time kill assay. Similar observations as these have also been reported with other *Euphorbia* species extracts by other researchers<sup>14</sup>. It has been observed that organic solvents extract better than water<sup>13</sup>. However a mixture of the two solvents yielded better results in this study.

## Conclusion

From this experiment crude extracts of *Euphorbia zeylanicum* plant were efficacious against some dermatophytes, yeasts and opportunistic pathogens which support its ethno medicinal uses as a broad spectrum herbal remedy. Further research on the fractions of these plant extracts to reveal the actual bioactive compounds will be of great value. This plant might thereafter be used as treatment for infections caused by these fungi.

## References

1. Sultan LS, Kimaro CC, Amri E., Antifungal activity and phytochemical screening of different solvent extracts of *Euphorbia tirucalli*. J. Adv. Biol. & Biotech., 2016; 7(1):1-9.
2. Ogunnusi TA., Antifungal activity of extracts of *Euphorbia kamerunica* PAX.IOSR J. Appl. Phys. (IOSR-JAP)., 2015, 7(3):63-68.
3. Saravanasingh K, Frdrik PG, Ramamurthy M. A., study on antibacterial and antifungal activities of extracts of medicinal plant *Aegle marmelos*. Int. Journal of Adv. Res. Biol. Sc., 2016; 3(2):321-328.
4. Kirbag S, Pinar E, Fikriye Z, Guvenc AN., Antimicrobial Activities of Some *Euphorbia* species. Afr. J. Trad. Compl. Alt. Med., 2013; 10(5):305-309.
5. Ahmad I, Khan AU, Bashir AC, Khalid HJ, Muhammad U, Muhammad A. et al. Antifungal and antispasmodic activities of the extracts of *Euphorbia granulate*. J. Med. Plants Res., 2012; 6(1):19-23.
6. Carter S, Eggli U., The CITES checklist of succulent *Euphorbia* Taxa (*Euphorbiaceae*) 2<sup>nd</sup> Edition Federal Agency of Nature Conservation Germany Bonn-Bad Godesberg; 2003.
7. Tarh JE, Okafor JI, Andiroegbu CU., Evaluation of extracts of *Coleus* species for antibacterial activity. Afr. J. Biotech., 2015, 14(2):125-132.
8. Tarh JE, Iroegbu CU. Evaluation of anti- fungal activity of *Coleus* species extracts. Int., J. Curr. Res. Biosc. Plant Biol, 2017; 4(1):131-138.
9. Tarh JE, Okafor JI, Andiroegbu CU., Evaluation of extracts of *Coleus* species for antibacterial activity, Afr. J. Biotech. 2015; 14(2):125-132.
10. Tarh JE, Iroegbu CU., Evaluation of anti- fungal activity of *Coleus* species extracts., Int. J. Curr. Res. Biosc. Plant Biol, 2017; 4(1):131-138.
11. Bhaskara R.K, V. Karthik, Elumalai EK. Srinivasan K, Kumar G., Antibacterial and Antifungal activity of *Euphorbia hirta* L Leaves: A comparative study., J. Pharm. Res., 2010; 3(3): 548-549.
12. Khan AM. Qureshi RA, Gillani SA, Ulla F., Antimicrobial activity of selected medicinal Plants of Margalla Hills Islamabad Pakistan., J. Med. Plants Res., 2011; 5(18): 4665-4670.
13. Lagnika L, Fantodji MHT, Sanni A., Phytochemical study and antibacterial Antifungal and antioxidant properties of *Bridelia ferruginea* and *Pteleopsis suberosa*., International J. Pharmaceuticals Sc. Res., 2012;3(7):2130-2136.
14. Gayathri A, Ramesh KV., Antifungal activity of *Euphorbia hirta* L Inflorescence extract against *Aspergillus flavus* a mode of action study., Int. J. Curr. Microbiol. Appl. Sc., 2013; 2(4):31-37.

\*\*\*\*\*