# BIOLOGICAL SCREENING OF *EUPHORBIA HELIOSCOPIA L.*

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**ABSTRACT**
The present research is preliminary biological screening of *Euphorbia helioscopia L.* (Euphorbiaceae). Dichloromethane and methanol extracts of the aerial parts of the plant were investigated for their antioxidant, antifungal, antibacterial and phytotoxic activities. Dichloromethane extract exhibited significant activity against *Fusarium solani* with 90 % Inhibition, where as the same extract also showed non-significant activity against *Salmonella typhi* and *Bacillus subtilis.* Methanolic extract has promising radical-scavenging activity in this assay. Both the extracts have non-significant phytotoxicity against *lemna minor*.

**Keywords:** *Euphorbia hellioscopia, Fusarium solani, Salmonella typhi, Bacillus subtilis.*

## INTRODUCTION

*Euphorbiaceae* is the largest family among the anthophyta, with 300 genera and 5000 species, sub cosmopolitan but with strong representation in humid tropic and subtropics of both hemispheres. The family *Euphorbiaceae* is represented in Pakistan by 24 genera of which 11 are not native (Nasir *et al*., 1986). Different species of *Euphorbia* are used for the treatment of various ailments such as skin diseases, gonorrhea, migraine, intestinal parasites and warts cures. The plant lattices have been used as fish poison, insecticide and as ordeal poison (Kinghorn *et al*., 1975). *Euphorbia* is stated to possess inflammatory, antiarthritic, antiamoebic, spasmolytic, antiviral, Hepatoprotective, and antitumor activity (Bani *et al*., 2000; Tona *et al*., 2000; Semple, *et al*., 2000; Shimura *et al*., 1990). The major classes of secondary metabolites present in *Euphorbia* species are alkaloids, terpenes, cyanogenic glycosides, glucosinolates, lipids and tannins (Hegnauer, *et al*., 1989). *Euphorbia helioscopia L.* whole plant has great medicinal importance, the leaves and stems are used as febrifuge and vermifuge (Wu *et al*., 1991).The oil from the seeds has purgative properties, the roots are used as anthelmintic and the seeds mixed with roasted pepper has been used in the treatment of cholera (Panda *et al*., 2004). In our previous study petroleum ether dichloromethane and methanol, extracts of *Euphorbia helioscopia L* were found inactive against *Claudosporium cucumerinum* plant pathogenic fungus (Chaudry *et al*., 2001). Objective of the study is to explore the potential of local medicinal plants regarding antioxidant, antifungal antibacterial and phytotoxic activities.

## RESULTS AND DISCUSSION

Dichloromethane and methanol extracts of the aerial parts of *Euphorbia helioscopia L* were studied for their antifungal, antibacterial, antioxidant and phytotoxic activities. Test fungi namely *Trichphyton longifusus, Candida albicans, Aspergillus flavus, Microsporum canis, Fusarium solani* and *Candida glabrata* were employed for fugitoxic effect of the extracts. Dichloromethane extract showed 90% Inhibition against *Fusarium solani*, at the concentration of 400 µg /ml for incubation period of seven days at 27ºC with reference to miconazole as standard. While methanol extract was found to be inactive (table 1). Antibacterial activity of the extracts was performed against *Eschericha coli, Bacillus subtilis, Shigella flexenari, Staphylococcus aureu, Pseudomonas aeruginosa* and *Salmonella typhi.* Both the extracts exhibited non-significant activity against *Bacillus subtilis* and *Salmonella typhi* at the concentration of 3 mg /ml (table 2). Antioxidant activity (free radical-scavenging properties) of both extracts was evaluated by thin layer chromatography (TLC) autographic assay method, using 2,2-Diphenyl-1-(2,4,6 trinitrophenyl) hydrazyl (DPPH) as spray reagent. Methanolic extract appeared as a yellow spot against purple background because of the components responsible for free radical-scavenging properties when tested at 100µg concentration. Where as dichloromethane extract did not respond to DPPH. Dichloromethane and methanol extracts of the aerial parts of *Euphorbia helioscopia L* showed non-significant phytotoxicity at concentrations of 1000 µg/ml, 100 µg/ml and 10 µg/ml against *Lemna minor.*

*Fusarium solani* is one of the most frequently isolated fungi from soil and plant debris. It is a host-specific pathogen for number of agriculturally important plants, including *pea, cucurbits, and sweet potato* and causes hyalohyphomycosis in humans (Anaissie *et al*., 1997). It is also associated with serious invasive mycoses in immunocompromised and immunosuppressed patients (Summerbell *et al*., 2002). The optimal treatment regimen for patients with disseminated infections has not yet been established, but rapid diagnosis and recovery of the neutrophil count seem to be essential for survival (Pujol *et al*., 1997). On the other hand oxidation is the major cause of degradation of food and materials. In food auto oxidation effects mainly lipids, proteins, colorants, and

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**Table 1**: Percentage inhibition of linear growth (mm) of test fungi by dichloromethane and methanol extracts of *Euphorbia helioscopia* L.

| Name of the Fungus | Linear Growth of Test Fungi (mm) | | | % age of Inhibition | MIC of Standard Drug | |
|---|---|---|---|---|---|---|
| | Dichloro-methane Extract | Methanol Extract | Control | | Name of the Drug | µg/ml |
| *Trichphyton longifusus* | 100 | 100 | 100 | 0 | Miconazole | 70.00 |
| *Candida albicans* | 100 | 100 | 100 | 0 | Miconazole | 110.80 |
| *Aspergillus flavus* | 100 | 100 | 100 | 0 | Amphotericin B | 20.00 |
| *Microsporum canis* | 100 | 100 | 100 | 0 | Miconazole | 98.35 |
| *Fusarium solani* | 10 | 100 | 100 | 90 | Miconazole | 73.25 |
| *Candida glabrata* | 100 | 100 | 100 | 0 | Miconazole | 110.80 |

**Table 2**: Zone of inhibition (mm) of test bacteria by dichloromethane and methanol extracts of *Euphorbia helioscopia* L.

| Name of Bacteria | Zone of inhibition (mm) | | Zone of inhibition (mm) of standard Drug |
|---|---|---|---|
| | Dichloromethane Extract | Methanol Extract | |
| *Eschericha coli* | - | - | 30 |
| *Bacillus subtilis* | 10 | 9 | 37 |
| *Shigella flexenari* | - | - | 36 |
| *Staphylococcus aureu* | - | - | 26 |
| *Pseudomonas aeruginosa* | - | - | 32 |
| *Salmonella typhi* | 13 | 11 | 30 |

flavor compounds. To over come oxidative damages antioxidant are added (Halliwell *et al*., 1995). Free radical and reactive oxygen species are consider to be harmful to human health and may cause cancer, coronary diseases, arteriosclerosis, inflammatory disorders, and diabetes, as well as involved in the process of aging (Halliwell *et al*., 1994). The results suggest that dichloromethane and methanol extracts having significant antifungal and antioxidant activity may consider for further isolation and identification of constituents responsible for antifungal and antioxidant activities.

## EXPERIMENTAL

***Plant material***

The aerial parts of *Euphorbia helioscopia L.* were collected from the surroundings of Bahauddin Zakriya University Multan Pakistan.The plant materials was identified by Prof. Dr. Altaf Ahmad Dasti Institute of Pure and Applied Biological Studies, Bahauddin Zakriya University, Multan. A voucher specimen is deposited in the Herbarium of the Institute.

***Extraction***

The air-dried plant material was ground and extracted successively with dichloromethane and methanol (thrice with each solvent) at room temperature occasional shaking for 24 hrs. Extracts were concentrated by Rotavapor-R20 at 35ºC.

***Antioxidant assay***

Reduction of 2,2-Diphenyl-1-picrylhydrazyl (=2,2-Diphenyl-1-(2,4,6 trinitrophenyl) hydrazyl; DPPH) Radical. TLC autographic assay: after developing and drying, TLC plates were sprayed with a 0.2%DPPH solution in methanol. The plates were examined after 30 min spraying. Active compounds appear as yellow spots against a purple background (Cendet *et al*., 1997).

***Antifungal assay***

Test fungi such as *Trichphyton longifusus, Candia albicans, Aspergillus flavus, Microsproum canis, Fusarium solani* and *Candida glabrata* were employed for preliminary screening. Extracts were dissolved in sterile DMSO to serve as stock solution. Sabouraud dextrose agar was prepared by mixing Sabouraud 4% glucose agar and agar agar in distilled water. Known amount of media was dispensed into screw capped test tubes. Test tubes containing media were autoclaved 121°C for 15 minutes. Tubes were allowed to cool to 50°C and the desired concentration of extract was added into non-solidified media. The tubes were allowed to solidify at room temperature. Each tube was inoculated with a 4 mm diameter piece of inoculum removed from a seven-day-old culture of fungi. All culture-containing tubes were inoculated at optimum temperature of 28-30°C for growth for 7-10 days. Culture was examined at least twice a weekly during the incubation. With no visible growth of microorganism is taken to represent the MIC of the test

sample which is expressed in ug (Atta-ur-Rhman *et al*., 2001).

***Antibacterial assay*** (Agar Well Diffusion method)
The Petri plates are prepared with an inoculated media. Three wells of 8 mm diameter on one plate are cut with a borer and sealed with a drop of inoculated sterile media. All the solutions i.e., the extract, solvent and reference standard (Imipenum 10µg/disc.) was poured into their respective well by sterilized pipette. The petri dishes were incubated at 37°C for 24-48 hrs. Zones of inhibition were measured with vernier caliper (Atta-ur-Rhman *et al*., 2001).

***Phytotoxicity bioassay***
Prepared inorganic medium of 5.5-6.0 pH attained with KOH pellets.10 vials per dose 500, 50, 5 and control were prepared.15 mg of the extract was dissolved in 15 ml of the solvent. 1000, 100 and 10µl of solution to vials for testing allow the solvent to evaporate overnight. 2ml of medium was added in each vial containing a single plant a rosette of three fronds. Placed the vials in a glass dish filled with 2cm of water, sealed the container with stopcock grease and glass plate. Placed the dish along with vials in growth chamber for seven days at 25°C under fluorescent and incandescent light. Count the number of fronds per vials on day 3 and 7.Analyzed the data as percent of control with ED 50 computer program (Atta-ur-Rhman *et al*., 2001).

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