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Antifungal activity of *Euphorbia antiquorum* L. latex an *in vitro* study

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Abstract

Medicinal plants are important source of potentially useful structures for the development of novel chemotherapeutic agents. The present study was aimed to evaluate the antifungal effect of methanolic latex extract on some human pathogenic fungi. The inhibitory effect of the methanolic extract of *Euphorbia antiquorum* L. was tested against *Candida albicans*, *Candida cruzi*, *Candida tropicalis*, *Candida parapolisis* and *Aspergillus* sps using disc diffusion method. The methanolic extract of *Euphorbia antiquorum* L. was subjected to qualitative phytochemical screening for the presence of bioactive ingredients. The latex extract showed the presence of many biologically active molecules such as alkaloids, cynogenic glycosides, phenols, flavonoids and terpenoids. The latex extract shows significant zone of inhibition in dose dependent manner. This study scientifically supports the usage of latex as a remedy for various superficial fungal infections in traditional medicine.

Keywords: Antifungal activity, Latex extract, *Euphorbia antiquorum*.

1. Introduction

Medicinal plants are the back bone of traditional remedy. India is very rich in natural resources and the knowledge of traditional medicine and the use of plants as source of new drugs is an innate and very important component of healthcare system. However very little information is available about many useful herbs as experimental data. In recent years secondary plant metabolites have been extensively investigated as a source of medicinal agents. Thus it is anticipated that phytochemicals with good antimicrobial activity will be used for the treatment of microbial infections. According to [1], the success story of chemotherapy lies on the continuous search of new drugs to counter the challenges posed by resistant strains of microorganisms. Studies indicate that in some plants there are many substances such as peptides, tannins, alkaloids, essential oils, phenols and flavonoids among other compounds which could serve as sources for antimicrobial production. These substances or compounds have potentially significant therapeutic application against human pathogens including bacteria, fungi and viruses [1, 2]. The development of microbial resistance to the available antibiotics had led researchers to investigate the antimicrobial activity of medicinal plants [3]. There is a continuous and urgent need to discover chemical structures and novel mechanisms of action for new and reemerging infectious diseases [4]. Therefore are increasingly turning their attention to ethno-medicine looking for new leads to develop more effective drugs against microbial infections [5] and this had led to the screening of several medicinal plants for potential antimicrobial activity [6]. Based on this the present study was aimed to evaluate the antifungal activities of *Euphorbia antiquorum* latex extract against prominent human pathogenic fungi by disc diffusion method. The latex extract has also been qualitatively analyzed for the presence of different phytochemicals.

Materials and Methods

Plant material

The plant material used for the collection of latex was *Euphorbia antiquorum* L.

Kingdom: Plantae

Order: Malpighiales

Family: Euphorbiaceae

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Genus: *Euphorbia*
Species: *antiquorum*

Collection and preparation of *Euphorbia antiquorum* latex extract

Latex samples were collected early in the morning from each plant by nipping the leaves and stem or by incision of the trunk and branches of the plant and allowing the latex to drain in clean glass tubes separately, brought to the laboratory and kept in the refrigerator till use. The collected latex was mixed with methanol in the ratio of 1:9 (10%) and centrifuged at 3500 rpm for 5 minutes and the supernatant was used for their antifungal activity and phytochemical analysis.

Antifungal Assay

Microorganisms used

Pure strains of fungi like *Candida albicans* Berkhout, *Candida cruzi* Berkhout, *Candida tropicalis* Berkhout, *Candida parapsolisis* and *Aspergillus* sps. were procured from the IMTECH, Chandigarh and used for the assay. Antibiotic Flucanazole was used as the standard for antifungal study.

Disc Diffusion method

The fungal strains were cultured in Sabouraud's Potato Dextrose broth separately for 72 hrs. The antifungal activity was performed following disc diffusion method [7]. The sterile discs (5 mm in diameter; Whatmann No.1 filter paper) were dipped in different concentrations of latex extract of *E. antiquorum* (25, 50, 75 and 100µl/ml) and were placed over the spreaded agar media containing the fungal inoculum. The Dextrose agar plates used for antifungal tests were incubated at 27°C for 72 hours. Flucanazole was used as a positive control for antifungal tests. The experiment was repeated three times and the mean values were calculated. Antifungal activity was determined by measuring the diameter of the zone of inhibition surrounding fungal growth.

Phytochemical analysis

The *E. antiquorum* latex sample was analyzed for the phytochemical composition by qualitative method using standard protocols [8, 9].

Alkaloids

Portion of the latex was treated with few drops of aqueous solution of hydrochloric acid and 0.5ml of Mayer's reagent. Formation of white precipitate indicated the presence of alkaloids.

Cynogenic Glycosides

To 250µl of the latex, equal volume of cold concentrated sulphuric acid was added. Formation of intense colour indicates the presence Cynogenic glycosides.

Phenolic Compounds

Phenolic compounds of latex were detected by folin ciocalteau reagent. A portion of the latex was mixed with few drops of diluted folin ciocalteau reagent and aqueous solution of sodium carbonate mixture and was allowed to stand for 10 min. Formation of gray colour indicated the presence of phenol groups.

Flavonoids

Few drops of 1% aluminium solution was added to a portion of the latex. Yellow colouration indicated the presence of flavonoids.

Terpenoids

A red to purple color formation indicated the presence of terpenoids when a chloroform portion of latex was treated with an equal volume of concentrated sulphuric acid.

Tannins

A portion of latex was mixed with few drops of 0.1% ferric chloride and observed for brown green colouration which indicated the presence of tannins.

Saponins

To 0.5ml of latex, 5ml of distilled water was added. The solution was then vigorously shaken and observed for a stable persistent froth with honeycomb structure which indicated the presence of saponins.

Table 1: Antifungal activity of methanolic extract of *E. antiquorum* latex using Disc Diffusion method.

Name of the fungus tested	Different concentrations (µl/ml)				
	Standard	25	50	75	100
	Zone of inhibition (in mm)				
<i>Candida albicans</i>	16.5±0.95	10.0±0.82 (-39.39)	12.5±1.29 (-24.24)	13±0.82 (-21.21)	19.0±0.81 (15.15)
<i>Candida cruzi</i>	13.25±0.95	8.5±0.57 (-34.84)	11.75±1.25 (-11.32)	15.75±1.26 (18.86)	18.0±1.41 (35.84)
<i>Candida parapsolisis</i>	14.5±1.29	9.75±0.95 (-32.75)	12.5±1.29 (13.79)	14.25±1.70 (-1.72)*	17.25±1.70 (15.94)
<i>Candida tropicalis</i>	16.0±0.81	10.25±0.96 (-35.93)	12.25±0.95 (-23.43)	12.25±0.95 (-23.43)	15.75±0.96 (-1.56)*
<i>Aspergillus</i> sps.	Resistant	11.25±1.25	13.0±1.63	14.75±1.25	16.5±0.57

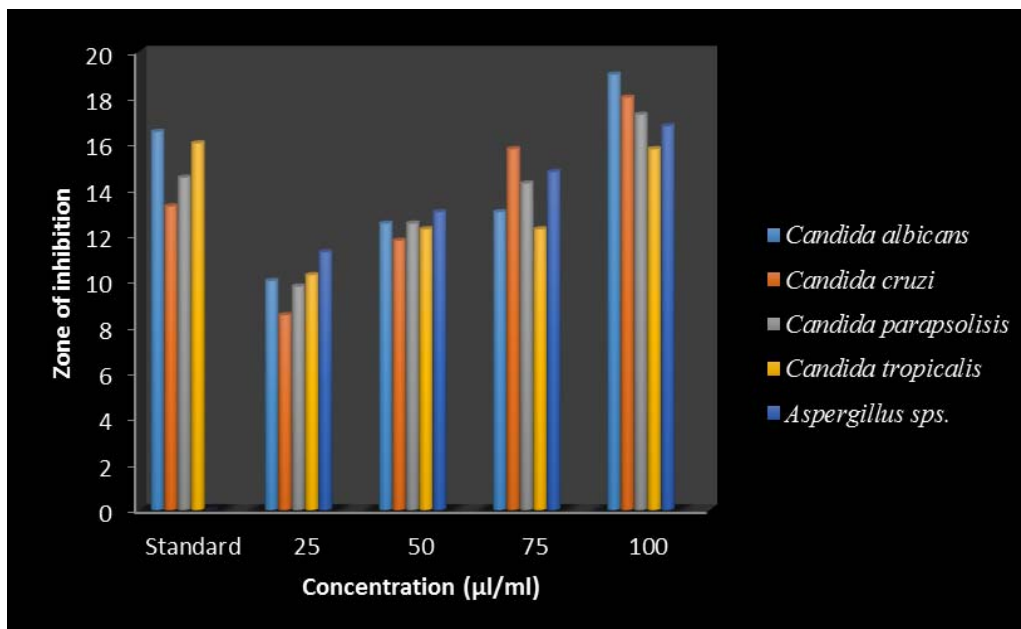


Fig 1: Showing the antifungal activity of methanolic extract of *E. antiquorum* latex using Disc Diffusion method

Table 2: Phytochemical investigation of *E. antiquorum* latex extract

Sl.No	Compounds	Observation
1	Alkaloids	Present
2	Cynogenic glycosides	Present
3	Phenols	Present
4	Flavonoids	Present
5	Terpenoids	Present
6	Tannins	Absent
7	Saponins	Absent

Result and Discussion

Plants are important source of potentially useful structures for the development of novel chemotherapeutic agents. Plant extract has been used traditionally to treat number of infectious diseases including those caused by bacteria, fungi, protozoa and viruses [10]. Table 1 shows the antifungal activity of methanolic extract of *Euphorbia antiquorum* latex against six different fungal species. The antifungal potency of *Euphorbia antiquorum* latex extract was evaluated by the presence or absence of inhibition zone and zone diameters (mm). From the result it is evident that the methanolic extract of *Euphorbia antiquorum* showed a maximum inhibitory zone in a dose dependent manner. Table 2 shows the presence of phytochemicals in the latex of *Euphorbia antiquorum*. The latex of *Euphorbia antiquorum* showed the presence of phytochemicals like flavonoids, tannins, alkaloids, phenols and cynogenic glycosides. This phytochemicals present in the latex of *Euphorbia antiquorum* were responsible for antimicrobial activity.

Methanolic extract of *Calotropis procera* latex and leaves have demonstrated strong inhibitory effect on the test microorganisms and the inhibitory effect was more pronounced in the latex extract [11]. The presence of biologically active compounds in the plant extracts could be correlated to the antifungal effects of substances known to possess activity [12]. Tannins have been found to form irreversible complexes with proline rich protein [13] resulting in the inhibition of cell protein synthesis. Alkaloid which is one of the largest groups of phytochemicals in plants having

amazing effects on humans and this had lead to the development of powerful painkiller medications [14]. Flavonoids another constituent exhibits a wide range of biological activities like antimicrobial, cytostatic and antioxidant properties [15].

Thus they can be used in sore and wound healing, as ear drop for boils and treatment of boils they can also be used in the treatment of infectious diseases caused by resistant microbes on the basis of the results obtained it can be concluded that crude extracts of *Euphorbia antiquorum* exhibit significant antifungal activity and properties that support folkloric use in the treatment of some diseases as broad spectrum antimicrobial agents.

Conclusion

The remarkable fungicidal effects of *Euphorbia antiquorum* latex extract suggest that the latex may be a useful source for the development of novel antifungal agent against pathogenic fungi.

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