

## **In vitro anti-Candida activity of *Ficus lyrata* L. Ethyl acetate latex extract and Nystatin on clinical Isolates and Standard strains of *Candida albicans***

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**Abstract.** In this study, the antimicrobial activity of ethyl acetate latex extract of *Ficus lyrata* L. and Nystatin on 65 clinical isolates of *Candida albicans* from Vulvovaginal candidiasis and standard strain of *C.albicans* ATCC 5027 were studied. The plant extract was obtained from Iranian commercial company. Inhibitory effects of the crude extract analyzed by using the disk diffusion technique (Bauer et al., 1966). The obtained results showed that *Ficus lyrata* L. extract has inhibitory effect on clinical isolates and type strain of *C.albicans* in lower concentrations than Nystatin drug with the diameters of inhibition zones ranging from 22 to 26 mm and 30 to 32 for clinical isolates and standard strains of *C.albicans*, respectively. The diameter of inhibition zones for Nystatin was between 16 to 20 mm and 21 to 24 mm for standard strain and clinical isolates of *C.albicans*, respectively. Based on the data analysis (Macro dilution broth method), the best MIC of *Ficus lyrata* L. ethyl acetate latex extract on clinical isolates and type strain of *C.albicans* were 25 mg/ml and 2.5 mg/ml, respectively. The best MIC of Nystatin on clinical isolates and type strain of *C.albicans* were 36 mg/ml but MIC of combination of both showed more potency than Nystatin alone (0.05mg/ml), which is a synergistic effect. The GC/MS analysis of latex showed that extract contains alkaloids, flavonoids, phenols, Tannins, terpenoid. The present study forms the basis for further investigations to isolate active components, elucidated the structures and evaluates them against wider range of microbial strains with the goal to find new the therapeutic principles.

**Keywords:** *Ficus lyrata*, *Candida albicans*, Nystatin, Latex extract

### **1. Introduction**

Today's use of medicinal plants and bioactive phytochemicals worldwide and our scientific knowledge of them comprise the modern field of the "phytosciences." The phytosciences have been created from the integration of disciplines that have never been linked before, combining diverse areas of economic, social, and political fields, chemistry, biochemistry, physiology, microbiology, medicine, and agriculture. The field is unique among the biomedical sciences in that instead of testing a hypothesis, in the phytosciences researchers try to determine whether plants commonly used in traditional medicine brings benefits for health and, if so, what their mechanisms of action are. Despite the common belief that phytochemicals are safe, they all have inherent risks just like synthetic compounds. Thus it is within the scope of the phytosciences to elucidate side-effects, appropriate doses, identify bioactive phytochemicals and ways of extraction and conservation. Besides these, legal aspects regarding regulation of the prescription and commercial sale of medicinal plants are a matter of debate all around the world. The varied regulations in different jurisdictions regarding the prescription and sale of these products add confusion to the formal use of phytochemicals. Several phytochemical surveys have been published, including the random sampling approach which involved some plant accessions collected from all parts of the world. The major chemical substances of interest in these surveys have been the alkaloids and saponins, however, other diverse groups of naturally occurring Phytochemicals such as flavonoids, tannins, unsaturated sterols, triterpenoids, essential oils, etc. have also been reported. Our approach involved the collection, identification, extraction and phytochemical

evaluation of extracts derived primarily from a random selection of commonly occurring native plants. Aim of this research was preliminary phytochemical screening, bioactivity determination and finally MIC of ethyl acetate latex extract obtained from *Ficus lyrata* Linn. against standard strain and clinical isolates of *Candida albicans*. Analysis was performed using agar disk diffusion and the broth macrodilution assay respectively.

## 2. Botany

*Ficus lyrata* L. belongs to family Moraceae. *Ficus lyrata* Linn. (Syn: *Ficus sycomorosa*; family: Moraceae) is commonly referred as “Fig” . A small or moderate sized deciduous tree, 15-30 ft high with broad ovate or nearly orbicular leaves, more or less deeply 3-5 lobed, rough above and pubescent below; fruits axillary, usually pear shaped, variable in size and color. The fig plant is considered to be a native of carica in Asia and is grown in nearly all tropical and sub-tropical Countries. It is now cultivated chiefly in the Mediterranean region, from Turkey in the east to Spain and Portugal in the west; it is also grown commercially in parts of U.S.A. and Chile and, to a small extent, in India, Arabia, China and Japan(13). Its fruit, root and leaves are used in the native system of medicine in Different disorders such as gastrointestinal (colic, indigestion, loss of appetite and diarrhea), respiratory (sore throats, coughs and bronchial problems), inflammatory and cardiovascular disorders. Fig has been traditionally used for its medicinal benefits as metabolic, cardiovascular, respiratory, antispasmodic and anti-inflammatory remedy. *F. lyrata* has been reported to have numerous bioactive compounds such as arabinose,  $\beta$ -amyrins,  $\beta$ -carotenes, glycosides,  $\beta$ -setosterols and xanthoxol (2, 7, and 12).Preliminary identification of the plant was done in the field by a botanist. Herbarium specimens were prepared and photographs were taken to aid in the confirmation of the identity of the plant. Voucher specimens were deposited in the Herbarium of the botany department, Islamic Azad University of Lahijan, where identity of the plants was confirmed by comparison with available voucher specimens.

## 3. Plant extracts and chemicals

*Ficus lyrata* L. crude extract (10.29 %w/w) were purchased from (Barij essence co. Tehran-Iran). All other chemicals used in this study were obtained from Sigma Chemical Co. (St Louis, MO, USA).

## 4. Extraction and isolation

100 g of powdered Latex was extracted with methanol (three times with a total of 800 ml of methanol) for 24 h at room temperature. The methanol extracts were collected, filtered and dried under vacuum to yield 7.1 g. The dried methanol extract was suspended in 10% aqueous methanol and was successively extracted with and ethyl acetate. The obtained extract, in addition to the aqueous solution remaining after extraction, were filtered and concentrated under vacuum to receive 0.5 g of ethyl acetate. Each extract was dissolved in methanol and refrigerated for further experiments.

## 5. Organisms

Standard strain of *Candida albicans* (ATCC 5027) obtained from ATCC, Fairfax, VA, USA, was grown and maintained on SDA agar slants.

## 6. Inoculum

Saboraud dextrose agar (SDA; Biotec Laboratories Ltd.UK) was used to prepare the culture medium according to the manufacturer's directions. *Candida albicans* ATCC5027 (provided by IROST organization in Iran) was aseptically inoculated on petridishes containing autoclaved, cooled and settled medium. The petridishes were incubated at 31°C for 48 h to give white round colonies against a yellowish background. These were aseptically sub-cultured on SDA slants *Candida albicans* colonies from SDA slants were suspended in sterilized 0.9% sodium chloride solution (normal saline), which was compared with 0.5 McFarland solution. The microbial suspension (1 ml) in normal saline was added to 74 ml of sterile medium, kept at 45°C, to give concentration of  $2 \times 10^7$  cells/ml.

## 7. Antimicrobial assay on *Candida albicans*

Antimicrobial activity of crude extract of the plant sample was evaluated by the paper disc diffusion method. For the determination of anti-*Candida* activity, isolates of *Candida albicans* were first adjusted to the concentration of 10<sup>6</sup> cfu/ml. Cultures of *Candida albicans* were suspended in sterile solution of 0.9% normal saline and all the cultures were inoculated onto saboraud dextrose agar plates. Sterile filter paper discs (diameter 13mm for yeasts) impregnated with 100µl of extract dilutions were applied over each of the culture plates previously seeded 10<sup>6</sup> cfu/ml cultures of yeast. Isolates of *Candida albicans* were then incubated at 37°C for 18 h. Paper discs impregnated with 20µl of Nystatin as standard antimicrobials were used for comparison. A negative control disc was impregnated with 100µl of sterile normal saline used in each experiment. Antimicrobial activity was determined by measurement of zone of inhibition around each paper disc. For each extract three replicate trials were conducted against each organism.

## 8. Results & Discussion

The phytochemical analysis exhibited that *F. lyrata* latex extract contains alkaloids, flavonoids, coumarins, saponins, and terpenes (4, 17). Some phenolic compounds, with reported pharmacological properties have already been isolated from fig leaves, namely furanocoumarins like psoralen and bergapten, flavonoids like rutin, quercetin, and luteolin, phenolic acids like ferrulic acid, and also phytosterols like taraxasterol (5, 17). Phenolic compounds constitute an important class of phytochemical which possesses diverse biological activities like astringent, antioxidant, anticancer, anti-inflammation, and antibacterial activity, etc (9, 14, and 17). The phytochemical screening of extract showed the presence of major derivatives and their results have summarized (Table 1). Phytochemical screening of crude extract showed the occurrence of alkaloids, flavonoids, phenols, Tannins, terpenoid. In this study; we demonstrated that ethyl acetate latex extract contains substances which have anticandidal effects. Mi-Ran Jeong et al. (2009) showed some flavonoids compounds. Also, synergistic effects of the MeOH extract with ampicillin or gentamicin combination against oral bacteria were presented in this research. Ahmad et al. (2001) demonstrated that only glycosides and saponins extracted from *F. lyrata* leaves using alcohol as solvent had biological effects but they had no effects on *C. albicans*, *S. aureus* and *E. coli* (Ahmad et al., 2001), compared to our study, latex extract are more active on human pathogenic yeasts and standard strains, respectively. These results may suggest that ethyl acetate extract of *F. lyrata* latex possess compounds with antibacterial and anticandidal properties which can be used as antimicrobial agents in new drugs for therapy of infectious diseases. Methanolic, hexanoic, chloroformic and ethyl acetate extracts of *Ficus lyrata* latex were investigated for their in vitro antimicrobial proprieties against five bacteria species and seven strains of fungi (Houda Lazreg Aref et al., 2010). In this study, the methanolic extract had no effect against bacteria except for *Proteus mirabilis* while the ethyl acetate extract had inhibition effect on the multiplication of five bacteria species (*Enterococcus faecalis*, *Citobacter freundii*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Proteus mirabilis*) (3,11). For the opportunist pathogenic yeasts, ethyl acetate and chlorophormic fractions showed a very strong inhibition (100%); methanolic fraction had a total inhibition against *Candida albicans* (100%) at a concentration of 500µg/ml. In agar disc diffusion method, the inhibition zone for Nystatin and crude extract were 16 to 20 mm, 21 to 24 mm for standard strain and clinical isolates of *C. albicans* and both 28 mm, respectively (Table 2). The best MIC of *Ficus lyrata* L. ethyl acetate latex extract on clinical Isolates and type strain of *C. albicans* were 20 mg/ml and 2.5 mg/ml, respectively. While, the best MIC of Nystatin on clinical Isolates and type strain of *C. albicans* were 0.5 mg/ml and both 0.05 mg/ml. In our comparative study Nystatin has a potent anti *C. albicans* effect. MIC of the extract had less potent than Nystatin, but MIC of combination of both showed more potency than Nystatin alone, which is a synergistic effect (Table 3). In fact, the present finding justifies the use of *F. lyrata* latex in traditional medicine for the treatment of various disease conditions whose symptoms might involve fungal infections and points to the importance of ethnobotanical approach as useful measure for the discovery of new bioactive compounds (Boukef, 1986). However, further research is needed to identify the active agents responsible for the anticandidal, antidermatophytic, and antibacterial activities of *F. lyrata* latex.

Table 1. Phytochemical screening of the crude extract of *Ficus lyrata*

<i>Organism</i>	<i>extract</i>
<i>Saponins</i>	-
<i>Alkaloids</i>	++
<i>Phenolics</i>	++
<i>Tannins</i>	+
<i>Terpenoids</i>	+
<i>Amino acids</i>	-
<i>Flavonoids</i>	++

(++) abundant, (+) present, (-) absent

Table 2. Anti-Candida activity of *Ficus lyrata* L. Ethyl acetate latex extract and reference antibiotic determined by the disc diffusion test

<i>Diameter of Zones of Inhibition (mm)</i>	<i>Nystatin</i>	<i>Crude Extract</i>
<i>C.albicans (standard strain)</i>	28	16-20
<i>C.albicans (clinical isolate)</i>	28	20-24

Table 3. Anti-Candida activity (MIC) of *Ficus lyrata* L. Ethyl acetate latex extract (mg/ml)

<i>Determination of MIC</i>	<i>Nystatin</i>	<i>Extract</i>
<i>C.albicans (St.strain&amp;Isolate)</i>	0.05mg/ml	0.05mg/ml
<i>C.albicans (Clinical isolate)</i>	0.5mg/ml	0.5mg/ml
<i>C.albicans (Standard strain)</i>	25mg/ml	2.5mg/ml

## 9. References

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