

Evaluation of Antifungal Activity of Methanol Extract of Acacia (*Anagalis Arvensis*) Leaves and Nystatin against *Candida Albicans* in Vitro

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Article information	Abstract
<p>Article history: Received: 20 Apr 2011 Accepted: 7 Sep 2011 Available online: 30 Dec 2012 ZJRMS 2013; 15(8): 39-41</p> <p>Keywords: Nystatin Extract Anagalis arvensis Candida albicans Minimum inhibitory concentration</p> <p>*Corresponding author at: Department of Clinical Bacteriology, Faculty of Basic Sciences, Islamic Azad University of Lahijan Branch, Lahijan, Iran. E-mail: lionface2006@yahoo.com</p>	<p>Background: <i>Anagalis</i> is the name of Scarlet Pimpernel, with about 20 to 25 species of flowering plants in the family <i>Myrsinaceae</i>. The common variety (<i>A. arvensis</i>) is a low-growing annual plant found in Europe, Asia and North America. In this Investigation, the inhibitory effects of methanol extract of <i>Anagalis arvensis</i> leaves and drug nystatin on clinical isolates and standard strain of <i>Candida albicans</i> (14053 PTCC) isolated from patients with vulvovaginal candidiasis were studied.</p> <p>Materials and Methods: In the laboratory study of 45 clinical isolates of <i>Candida albicans</i> was used and inhibitory effects of the extract were evaluated using by serial dilution Method. Chemical compounds of <i>A. arvensis</i> extract including alkaloids, glycosides, phenolic compounds, Terpenoids and amino acids were identified.</p> <p>Results: The results showed that the minimum inhibitory concentration (MIC) nystatin against clinical isolates and standard strain of <i>Candida albicans</i> were similar and drug nystatin revealed the better MIC against standard strain and clinical isolates of <i>C. albicans</i>. Methanol extract of <i>A. arvensis</i> were inhibitory on standard strain and clinical isolates of <i>C. albicans</i>. The minimum inhibitory concentration of extract in comparison with the drug nystatin was less while the composition of the growth inhibitory concentration was greater than drug solely.</p> <p>Conclusion: This study indicated that the antimicrobial activity of this plant extract was considerable compared to drug nystatin and it demonstrate a basis for further research to isolate the active components in a variety of microbial strains.</p>

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Introduction

Herbal medicine, especially infectious diseases are growing in recent years. More than 100 years, plants used for therapeutic purposes. History of using alternative medicine of the last time until the mid-twentieth century represents a reduction of herbal Medications until the 1940s and increase in 1980 was to re-use them. Today, a number of effective anti-fungal and anti-bacterial drugs are used to treat infectious agents; however, the genetic diversity caused by microbial pathogens, emergence of resistant strains and side effects of drugs, replacing them by antimicrobial drugs of plant origin is important. Considering the diversity of climate and diversity of flora and vegetation in Iran, identifying of the effective ingredients extracted from native plants to mass production have had a great importance. Biologically active compounds such as antioxidants, anti-microbial and anti-tumor agents in plants caused that from which the plants be used as herbal remedies and supplements, food preservatives. For centuries, plants have been used in various diseases such as hypertension, eczema and diarrhea [2]. Today, it has been recognized

that more than 50% of all drugs used in clinical cases form medicinal plants [3].

In a study on extracts of two kinds of plant species (*A. foemina* and *A. arvensis*) that from which are used traditionally in wound healing, it was found that both of them were showed anti-inflammatory activities [4]. Ali-shtayeh and et al in their study on the antifungal activity of extracts including *A. arvensis* against dermatophytes showed that among the most active extracts, *J. regia* with *A. arvensis* are shown more antifungal effects against the three most common dermatophyte [5]. In another study in the evaluation of the antimicrobial activity of some weeds in India, among other extracts, aqueous and methanol extracts of these species compared with the number of standard antibiotics were indicated significant antimicrobial activity against gram-negative and gram-positive bacteria pathogens [6]. In this study, the efficacies of the methanol extract of Acacia leaves, native to northern Iran, due to its intrinsic antimicrobial activity against standard strain and clinical isolates of *C. albicans* investigated.

Materials and Methods

The agar disc diffusion method was employed to determine the antimicrobial activity of the crude extract with measuring of diameter of growth inhibition zones. To show effects of Anti-Candida activity, The yeast suspension at concentration 10^6 cfu/ml were adjusted and 100 μ l of suspension inoculated onto Sabouraud Dextrose Agar (oxoid) plate and incubated at 37°C for 24 h. Filter paper discs (13 mm in diameter) placed on the inoculated plates. Impregnated Paper disks with 20 μ l of various concentrations of the extract were diluted using DMSO solution. Nystatin drug was used as a positive control at concentration 30 μ g/ml. Then; culture media inoculated with yeasts incubated at 37°C for 24-30 hr. Diameter of growth inhibition zones around each paper disc was measured by caliper. Each experiment was performed using repeated twice.

Determination of minimum inhibitory concentration (MIC): To determinate MIC of methanol extract *C. albicans* were performed using broth dilution assay. 2 ml of Sabouraud Dextrose Broth and one loopful of yeast suspension was added to 0.5 ml of various concentration of the plant methanol extract (0.005–20 mg/ml). This method was used for both of clinical isolates and standard strain in combination. After incubation, microbial growth was compared with turbidometry.

Statistical Analyses: The comparison of average zone diameters and the evaluation of extract antimicrobial effects were analyzed by SPSS-11.5 and *t*-test. In this experiment, *p*-Value was statistically significant ($p < 0.05$).

Results

The obtained results of phytochemical combination of crude extract showed that glycosides and saponins compared to phenolics, Alkaloids, terpenoids and Amino acids were in more proportions ($p = 0.045$) (Table 1).

Table 1. Phytochemical compositions of the crude extract of *A. arvensis*

Chemical constituents	Crude extract
Saponins	++
Alkaloids	+
Phenolic	+
Tannins	-
Terpenoids	+
Amino acids	+
Glycosides	++

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

In agar disk diffusion diameter of growth inhibition zones of crude extract were obtained from 20 mm till 24

Table 2. Anti-Candida activity of *A. arvensis* L. Methanol extract

Microorganism	Antimicrobial activity		Nystatin		Drug & Extract (MIC)* (μ g/ml)
	MIC (μ g/ml)	†Extract Bioactivity (mm)	MIC (μ g/ml)	Bioactivity (mm)	
<i>C. albicans</i> (Standard strain)	0.25	23	0.3	20-24	-
<i>C. albicans</i> (Clinical isolate)	0.25	23	0.4	18-22	-
<i>C. albicans</i> (Standard & Isolates)	-	-	-	-	0.05

mm and 18 mm till 22 mm for clinical isolates and standard strain, respectively however, this result for nystatin compared to clinical isolates and standard strain were the same and equal to 20 mm (Table 2). The best result of MIC were obtained for methanol extract of *A. arvensis* against pathogenic isolates and standard strain (0.3 and 0.4 mg/ml and Nystatin, 25 mg/ml), respectively ($p = 0.0255$).

In contrast, to both extract and drug combinations were indicated additional effect equal to 0.05 mg/ml with each other ($p = 0.0135$). In this study, the phytochemical composition of crude extract of Acacia plant showed that the extract obtained from the leaves of this plant contains alkaloids, saponins, flavonoids, terpenoids and phenolics. It seems that one or all of these components of plant is responsible for its antifungal effects. Phenolic compounds are an important class of phytochemical compounds containing diverse biological activities. This includes astringent, antioxidants, anti tumor, anti-inflammatory, antibacterial properties and etc [3, 8, 9].

The saponins are also the materials with toxic effects and hemolytic activity. Saponins are glycosides with terpenoids and/or estradiol based containing of surface active properties. In this study, it was found that the Acacia drug is highly effective against yeast. According to obtained MIC from this plant in specified concentrations was indicated less effectiveness rather than nystatin drug. Yet, composition of the extract and nystatin MIC enjoys higher fungicidal activity.

Discussion

In recent years many studies to evaluate the antimicrobial effects of essential oils and extracts have been indicating the ability of these compounds to inhibit the growth of pathogenic and spoilage microorganisms in food. Since, these compounds are natural and in many cases containing other existing health compounds, it has been emphasized for protecting of human health. Viewpoint of botany, medicinal plants with therapeutic and nutritional active compounds have always been the case.

Among this, overuse of antibiotics has caused the increasing resistance of bacteria against the drugs. On the other hand, the indiscriminate use of antibiotics, it is often associated with adverse effects on the human body. Since some plants with antimicrobial effects have been recorded in pharmacopoeia of the drug in Iran, it can be used to deal with some specific pathogenic microbes and safe to replace with some antibiotics [10].

In a study conducted on 22 plant extracts used in traditional medicine in Palestine, they showed anti-fungal activity against *Trichophyton mentagrophytes*, *Microsporium canis* and *Trichophyton violaceum*. Extracts reduced colony growth compared with the control drug ($p < 0.05$). In another study it was demonstrated that the most active among the tested plant extracts belonged to *A. arvensis*, *C. spinosa*, *J. regia* and *Rata chalapensis* [11]. Also, the sensitivity of dermatophytes significantly different than herbal extracts. The findings of the acacia plant extract used in traditional medicine to treat various diseases and their symptoms can be indicative of fungal infections is justified. It is important that traditional methods using herbs can be used to discover new strategies for new biologically active compounds. Shoji and et al. showed that the aqueous and methanol extracts of this plant contain saponins and flavonoids [12]. However, the need to further studies to identify the active agents responsible in other biological and pharmacological activities of this plant is necessary [13, 14]. Also, it is good that other antimicrobial activity of plant extracts and essential oil and other areas of native

species from view of the chemical composition of contents and their clinical efficacy must be investigated. In the future, it is recommended, researches on appropriate ratios of the two compounds are used to extract or oil with antibiotics in order to treatment of skin infections resistant to types of yeast and dermatophytes.

Acknowledgements

The authors appreciated the financial supports of Research Deputy and management of Microbiology Department, Dr. Faezi and respected experts and also Chemistry Department Mrs. Asadi and Alipour in order to successfully implement the project number 72 by M.R.M Khoshkholgh-Pahlaviani in Islamic Azad University; Lahijan branch.

Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

The authors declare no conflict of interest.

Funding/Support

Islamic Azad University of Lahijan Branch.

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Please cite this article as: Khoshkholgh-Pahlaviani MRM, Massiha AR, Issazadeh K, Bidarigh S, Ghihi M, Ramtin M. Evaluation of antifungal activity of methanol extract of Acacia (*Anagalis arvensis*) leaves and Nystatin against *Candida albicans*, in Vitro. *Zahedan J Res Med Sci (ZJRMS)* 2013; 15(8): 39-41.