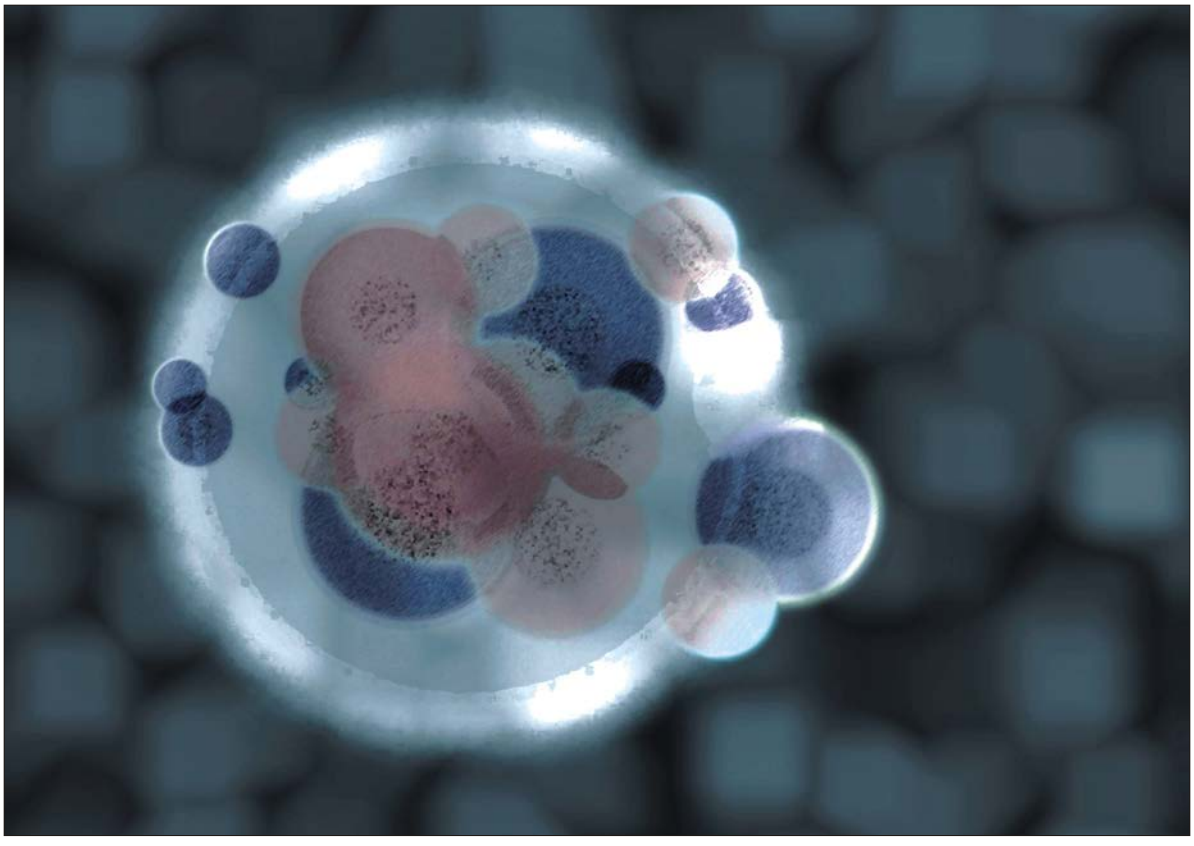

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Anti Yeast Activity of Some Plants Used in Traditional Herbal-medicine of Iran

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Abstract: This is the first report showing the anti-yeast activities of different Iranian plants. Plants used in herbal-medicine were collected from South-East regions of Iran. Methanolic extracts were prepared and evaluated in Agar well-diffusion test against three yeast species of *Saccharomyces cerevisiae*, *Candida albicans* and *C. utilis*. From 48 plant species in 34 families, 26 species in 20 families showed inhibitory effect at least against one yeast species at 20 mg ml^{-1}. Plants with high anti-yeast activity included *Alpinia officinarum*, *Chrozophora verbasafalia* , *Cinnamomum zeylanicum*, *Dianthus coryophyllus*, *Helleborus nigra*, *Heracleum persicum*, *Myrtus communis*, *Terminalia chebula* and *Trachysermum copticum* which were effective mostly against *C. albicans* and *C. utilis*. No plant showed high level of activity against *S. cerevisiae*. The lowest MIC was observed in *M. communis* and *T. chebula* as 0.31 mg ml^{-1}. Active extracts retained their activity at room temperature in both DMSO: methanol (1:1, v/v) solvent and lyophilized state up to 18 months.

Key words: Anti yeast, Iranian medicinal-plants, *Saccharomyces cerevisiae*, *Candida albicans*, *Candida utilis*

INTRODUCTION

Plants produce many secondary metabolites, many of them with antifungal activity. Well-known examples of these compounds include flavonoids, phenols and phenolic glycosides, unsaturated lactones, sulphur compounds, saponins, cyanogenic glycosides and glucosinolates[1,2]. Clinically, antimicrobial therapy is going through a crisis due to the rapid development of resistance to existing antimicrobial agents[3]. Twenty years ago, *C. albicans* was commonly regarded as little more than culture contaminant; however, because of developed antimicrobial-resistance, in less than 2 decades this organism has become a major human pathogen[4]. The present study was conducted to investigate anticandidal activity of plants which are used in Iranian Folkloric Medicine (IFM). Herbal remedies play a fundamental role in traditional medicine in rural areas of Iran where they are often the therapeutic treatment of choice as antiseptic, anti-inflammatory and in treatment of infectious diseases including candidiasis and dermatophytes. Some Iranian researchers[5,6] have previously reported antibacterial activity of few of these plants. Several workers have investigated anticandidal activity of plants of special regions too. Cavin *et al.*[7] screened 204 crude extracts of Indonesia plants on *C. albicans* and found nine actives. Crockett *et al.*[8] noticed anticandidal activity in *Cassia alata* and expressed that water extract of the plant is used in Ivory Coast, West Africa to treat fungal infections caused by *C. albicans* and dermatophytes. Two yeast species used in this study were chosen primarily based on their importance as opportunistic human-pathogens as *Saccharomyces cerevisiae,* commonly known as brewer's yeast, has been reported as the cause of both superficial and invasive infections[9] and *Candida albicans* while naturally occurring in the intestinal flora, can cause oral thrush and systemic infections. *C. utilis*, nonpathogenic yeast also incorporated into the screening survey in order to attain more comprehensive conclusion about the anti-yeast spectral activity of the plants.

MATERIALS AND METHODS

Plant material: Fifty plant samples including 48 species belonging to 34 families, being used in herbal-medicine in rural regions, were collected from different areas in South-East regions of Iran guided by local healers and knowledgeable villagers and identified by Mrs. P. Rashid Farrokhi in the Herbarium of Plant Systematic Laboratory of the College of Agriculture, Bahonar University of Kerman, Iran, where voucher specimens of plants were deposited. Information on the therapeutic properties of the plants was gathered by participating in domestic activities and interviewing on those used for therapeutic treatments as antiseptic, anti-inflammatory and in treatment of infectious diseases including candidiasis and dermatophytes. According to the information gathered about the ethnopharmacological usages, the plant organs used in this study were the same as used in herbal-medicine.

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Preparation of the extracts: Dried plant materials were ground with mortar and pestle or an electric mill. Powdered plant material (20-50 g of each sample) was soaked in 100-150 ml of methanol–water (90:10, v/v) at room temperature for 48 h. The extract was filtered through a Buchner funnel with Whatman filter paper number 1 and washed with methanol. The filtrate was evaporated to dryness under reduced pressure and freeze-dried for 24 h. The resulting crystalline or heavy syrup concentrates were kept at 4°C in the dark before use.

Preparation of test samples: The lyophilized methanol-extracts were dissolved in dimethyl sulfoxide (DMSO): methanol (1/1: v/v) solvent for the study of the antimicrobial activity. As a precaution not to miss trace amounts of antimicrobials, a relatively high concentration (20 mg ml^{-1}) of each extract was prepared for bioassays.

Test microorganisms: All of the plant extracts were assayed for anti-yeast activity against three registered yeast species which were obtained from the Persian Type Culture Collection, Tehran, Iran (PTCC). They included: *Saccharomyces cerevisiae* (PTCC No. 5052), *Candida albicans* (PTCC No. 5027) and *C. utilis* (PTCC No. 5065). The yeasts were grown at 30°C and stored at 4°C on Sabouraud dextrose medium and sub-cultured as needed.

Agar well diffusion assay: Plates of Sabouraud dextrose-agar media were seeded with a suspension of actively growing yeast cells. Suspension of approximately $1.5x10^8$ yeast cells ml^{-1} in sterile normal saline were prepared as described by Forbes *et al.*[10] and about 1.5 ml of it was uniformly seeded on nutrient media in 12x1.2 cm glass Petri dishes, left aside for 15 min and excess of suspension was then drained and discarded properly. Wells of 6 mm in diameter and about 2 cm apart were punctured in the culture media using sterile cork borers. A fixed volume of respective extracts were loaded in the wells using sterilized dropping pipettes and diffusion was allowed at room temperature for 2 h. After preincubation, the plates were incubated aerobically at 30°C for 24 to 48 h. Anti-yeast activities were determined by measuring Diameter of inhibition zones (DIZ) in mm. Each experiment was repeated thrice and the average values of antimicrobial activity were calculated.

Determination of minimum inhibitory concentrations (MIC): To measure the MIC values, concentrations of 10, 5, 2.5, 1.25, 0.62, 0.31and 0.15 mg ml^{-1} of the methanolic extracts were prepared in DMSO: methanol (1/1: v/v) solvent and assayed against the yeasts as mentioned earlier. The MIC was defined as the lowest concentration able to inhibit any visible yeast growth. All data represent average of three replicated experiments.

Test controls: Negative controls included use of solvent without test compounds, although no anti-yeast activity noted in the solvent employed for the test. Positive controls included use of common antifungal agent (Clotrimazole 1% topical solution, Pars Daru, Tehran, Iran) in a separate well of each plate in order to control the sensitivity of the test organism.

Estimation of longevity *in vitro* of the active extracts: To estimate the stability of the active extracts in both soluble and dry states, 20 mg ml^{-1} of each sample was prepared in DMSO: methanol (1:1, v/v) solvent and 20 mg dry samples were placed in small vials. These samples kept at room temperature and tested for anti-yeast activity against the most sensitive isolate at 14 days intervals up to 18 months.

RESULTS

Assay results of plants used in Iranian traditional herbal-medicine against three yeast isolates of *S. cerevisiae*, *C. albicans* and *C. utilis*, are listed in Table 1. Anti-yeast inhibitory effects of methanol extracts, indicated as mm of DIZ and estimated MIC as mg ml^{-1} are indicated. From 48 plant species in 34 families, 26 species in 20 families showed inhibitory effects against at least against one yeast species. The activities were rated into three classes as: low (DIZ<12 mm), moderate (DIZ=12 to <18 mm) and high (DIZ=18 mm or higher). Plants with high anti-yeast activity included *Alpinia officinarum*, *Chrozophora verbasafalia*, *Cinnamomum zeylanicum*, *Dianthus coryophyllus*, *Helleborus nigra*, *Heracleum persicum*, *Myrtus communis*, *Terminalia chebula* and *Trachysermum copticum* which were effective mostly against *C. albicans* and *C. utilis*. No plant showed high level of activity against *S. cerevisiae*. The lowest MIC was observed in *M. communis* and *T. chebula* as 0.31 mg ml^{-1}. Studies on longevity *In Vitro* showed that active extracts retained their activity at room temperature in both DMSO: methanol (1:1, v/v) solvent and lyophilized state up to 18 months.

DISCUSSION

Although Iran possesses a rich tradition in the use of medicinal plants and an outstanding floral diversity of

Table 1: *In vitro* screening results, indicated as mm of diameter of inhibition zones (DIZ), of anti-yeast inhibitory effects of methanol extracts of plants used in Iranian traditional herbal-medicine against three yeast isolates of *Saccharomyces cerevisiae*, *Candida albicans* and *C. utilis*. Plant species, families, plant organs-tested and estimated minimum inhibitory concentrations (MIC) as mg ml^{-1}, are indicated.

			a		b		c	
Plant Species	Plant Families	OT	DIZ	MIC	DIZ	MIC	DIZ	MIC
Alpinia officinarum	Zingiberaceae	RH	-	-	20	1.25	15	5.00
Amomum subulatum	Zingiberaceae	SE	-	-	-	-	14	5.00
Berberis vulgaris	Berberidaceae	FR	-	-	14	5.00	-	-
Borago officinalis	Boraginaceae	FL	-	-	12	10.00	-	-
Capsicum annum	Solanaceae	FR	10	10	10	10.00	14	5.00
Chrozophora verbasafolia	Euphorbiaceae	LE	-	-	21	1.25	13	5.00
Cinnamomum zeylanicum	Lauraceae	SB	-	-	22	1.25	18	2.50
Cuscuta epithymum	Convolvulaceae	SE	-	-	-	-	10	10.00
Dianthus coryophyllus	Caryophyllaceae	FL	-	-	24	0.62	20	0.62
Ephedra intermedia	Ephedraceae	ST	-	-	16	1.25	15	1.25
Foeniculum vulgare	Umbelliferae	RO	-	-	-	-	13	5.00
Helicteres isora	Sterculiaceae	FR	-	-	10	10.00	-	-
Helleborus nigra	Ranunculaceae	RO	-	-	24	0.62	10	10.00
Heracleum persicum	Umbelliferae	FR	-	-	24	2.50	10	10.00
Hyoseyamus niger	Solanaceae	SE	-	-	10	10.00	-	-
Myristica fragrans	Myristicaceae	SE	-	-	-	-	12	10.00
Myrtus communis	Myrtaceae	LE	-	-	24	0.31	22	0.62
Myrtus communis	Myrtaceae	SE	-	-	22	0.31	14	1.25
Pimpinella anisum	Umbelliferae	FR	-	-	10	10.00	15	5.00
Rubus idaeus	Rosaceae	LE	-	-	12	10.00	12	10.00
Salvia officinalis	Labiatae	FL	-	-	10	10.00	12	10.00
Semicarpus anacardium	Anacardiaceae	SB	11	10	10	10.00	13	10.00
Smilax china	Liliaceae	RO	10	10	15	5.00	18	5.00
Terminalia chebula	Combretaceae	US	-	-	31	0.31	17	1.25
Terminalia chebula	Combretaceae	RS	-	-	32	0.31	18	1.25
Thymus vulgaris	Labiatae	WP	-	-	15	5.00	14	5.00
Trachysermum copticum	Umbelliferae	FR	-	-	18	2.50	10	10.00
Zingiber officinale	Zingiberaceae	RH	-	-	10	10.00	17	1.25
Clotrimazole 1%	15	N/T	24	N/T	16	N/T		

a: *Saccharomyces cerevisiae*, b: *Candida albicans*, c: *C. utilis*. OT: plant organ tested, as FL: flower, FR: fruit, LE: leaves, RH: rhizome, RO: roots, RS: ripen seeds, SB: stem bark, SE: seeds US: unripe seeds, WP: whole plant. DIZ: diameter of inhibition zones (mm) at 20 mg ml^{-1}, MIC: Minimum inhibitory concentration (mg ml^{-1}). N/T: not tested.

vascular plants, little research has been done in the context of phytochemical leads for therapeutic use. The present study has clearly demonstrated that the medicinal knowledge held by the Iranian native people is relatively measurable in laboratory-based assays. It is clear that plants used by them for antimicrobial purposes show anti-yeast activities. The results of this initial screening on part of Iranian ethnobotany show that about half of the plants tested had some degree of anti-yeast activity. Frequency of *Candida albicans* infections has risen dramatically since the advent of antibiotics and the development of drug-resistant *C. albicans* is a major concern worldwide[11,12]. Kieren *et al.*[13] reported that Fluconazole-resistant *C. albicans*, a cause of oropharygeal candidiasis in patients with human immunodeficiency virus infection has recently emerged as a cause of candidiasis in patients receiving cancer chemotherapy and marrow transplantation. In their studies they found *C. albicans* isolates became resistant to fluconazole and amphotericin B after 2 weeks of antifungal drug exposure. Development of drug-resistant pathogens demands new strategies and the native people's ethnobotanical knowledge which has received less emphasis, is a valuable resource which should be utilized to advance health-oriented objectives.

Since the incidence of strains of *C. albicans* with multiple antibiotic resistances is increasing worldwide, it is of great importance to find effective treatments for infection by these pathogens. Novel, safe and effective compounds may be found through consultation with traditional healers or native people using herbal medicine. Certainly indigenous plants are reservoirs of novel antimicrobials; they would play important roles in providing us with such bioactives in future. Plants with high anti-yeast activity presented here or in similar studies would act as bedrocks for expanding our knowledge to attain new principles.

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