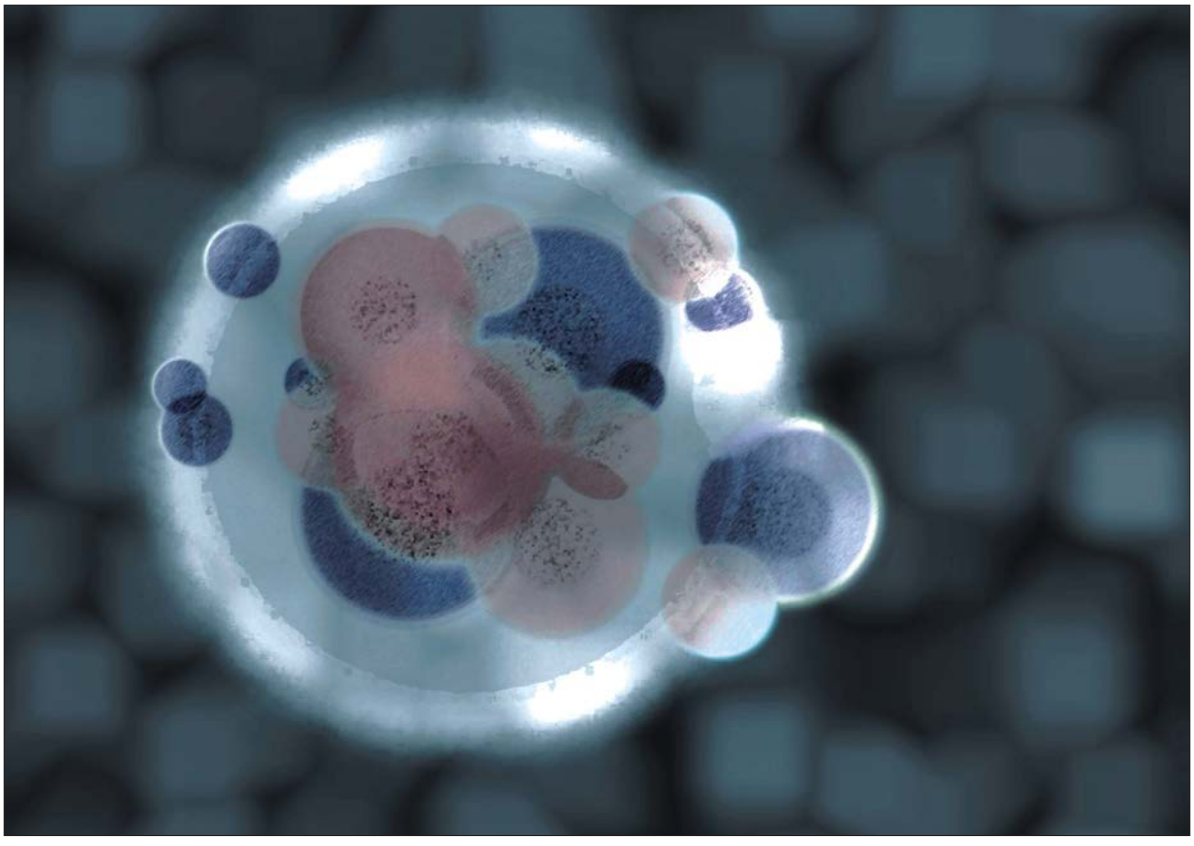

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Antimicrobial Activity of Some Medicinal Plants Against *Candida albicans*

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Abstract: Four extract amounts (200, 150, 100 and 50 mg mL^{-1}) from twelve medicinal plant species that belong to six genera (*Achillea*, *Salvia*, *Convolvulus*, *Plantago*, *Anthemis and Artemisia*) were tested against *Candida albicans*. The antimicrobial activity was carried out by using the hole-plate diffusion method. The effect of plant species, extract amounts and their interaction were highly significant. *Achillea santolina, Salvia dominica* and *Salvia officinalis* inhibited the growth of *Candida albicans* at all tested extract amounts. The extracts of (*Salvia spinosa*, *Convolvulus althaeoides* and *Plantago lanceolata*) showed no activity against *Candida albicans*.

Key words: *Candida albicans,* medicinal plants, antimicrobial activity

INTRODUCTION

Jordan has not less than 2000 wild plant species belonging to about 700 genera. Among these plants, as many as 485 species from approximately 99 plant families are categorized as medicinal plants. Many of them are under collection pressure and often used for many medicinal purposes, some examples are: *Artemisia, Achillea, Salvia, Anthemis* and many others[1-4].

These identified plants are herbs, shrubs or trees. They are distributed all over the country with a wide range from the Eastern desert to other parts of the country. Those plants are massively used by Bedouins or local people in folk medicine as hot or cold drinks, or chewed raw materials as fresh or dry.

Medicinal plants are a valuable natural resource and regarded as potentially safe drugs. They have been playing an important role in alleviating human sufferings by contributing herbal medicines in the primary health care systems of rural and remote hilly areas where more than 70% of population depends on folklore and traditional system of medicines.

Medicinal plants have been tested for biological, antimicrobial and hypoglycemic activity[5-7]. They have also tested for antiulcerogenic, antihelminthic, hepatoprotective, analgesic, antipyretic, antileishmania and insecticidal activities[8-12].

Due to the widespread and often indiscriminate use of antimicrobial drugs, many microorganisms have acquired resistance to specific antibiotic treatments and these strains are particularly evident in the hospital environment[13]. This has created immense clinical problems in the treatment of infectious diseases[14]. In addition to this problem, antibiotics are sometimes associated with adverse effects on host, which include hypersensitivity, depletion of beneficial gut and mucosal microorganisms, immunosuppression and allergic reactions[15]. Research on medicinal plants has increased and their antimicrobial activity has been screened in number of studies[3,16-21].

Because of the side effects and the resistance that pathogenic microorganisms build against the antibiotics, much recent attention has been paid to extract biological active compounds from plant species that used in herbal medicine. In many parts of the world, medicinal plants are used for their antibacterial, antifungal and antiviral activities. These plant extracts were used as a source of medicinal agents to cure urinary tract infections, cervicitis, vaginitis, gastrointestinal disorders[22] and skin infections such as herpes simplex virus type I[23].

Herbalists praise *Achillea* for its anti-inflammatory, antipyretic, carminative, astringent, antispasmodic, stomachic and bactericidal action. Traditional use of *Achillea* against serious illnesses in ancient Persia attracted the attention of researchers interested in studying this plant's therapeutic effects on the immune system.

The name *Salvia* is from the Latin salvere, meaning to heal or to be safe and unharmed. As a medicinal plant, *Salvia* has been reported to act as an bactericide and is used in mouthwashes and gargles. The plant is also used as a convulsant and antisecretory agent and as an antimicrobial, anti-inflammatory agent in treating oral cavity disease[24].

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Rovinsky and Cizadlo[25] found that the antimicrobial effects of the acetone extraction of *Salvia divinorum* inhibited the growth of the gram negative organisms *Citrobacter freundi, Escherichia coli* and *Pseudomonas aeruginosa* and one gram positive organism, *Bacillus subtilis*. However, the extract had no effect on the growth of *Alcaligenes faecalis* and *Pseudomonas fluorescens* (gram negative organisms) and *Micrococcus luteus, Staphylococcus aureus, Staphylococcus epidermidis* and *Streptococcus lactis* (gram positive organism).

Nascimento *et al.*[26] found that the extracts from *Salvia officinalis* and *Achillea millefolium* did not inhibit the growth of *Staphylococcus aureus*, *Salmonella choleraesuis*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Candida albicans*, *Proteus* sp., *Klebsiella pneumoniae* and *Enterobacter aerogenes*.

Tepe *et al.*[27] found that *Candida albicans*, *Candida krusei*, *Mycobacterium smegmatis*, *Acinetobacter lwoffii*, *Streptococcus pneumoniae* and *Clostridium perfringens* were the most sensitive microorganisms to the *Salvia cryptantha* essential oil, whereas *Salvia multicaulis* essential oil had pronounced activity against *Streptococcus pneumoniae*, *Candida krusei*, *Clostridium perfringens*, *Mycobacterium smegmatis*, *Candida albicans* and *Staphylococcus aureus*.

Plantain is the general name for several small herbs used medicinally because of their mucilaginous properties; *Plantago psyillium*, *Plantago ovata*, *Plantago major* and *Plantago lanceolata*, are representatives of the species. The plant has been traditionally used as a remedy against insect bites, toothaches, fevers, ulcers and wounds. Other medicinal applications of the plantain species have included use as an astringent, demulcent and diuretic. Extracts of common plantain have been reported to exhibit antibacterial activity[24].

Anthemis pseudocotula (chamomile) could be used as anti-diabetic, anti-tumor, antifungal, bactericidal[28]. *Artemisia herba-alba* (wormwood) could be used for treatment cough, stomach and intestine pains, eye diseases, hair pomades and acts as anti-pyretic, hypoglycemic, anti-infections, antibacterial[28-30].

Mahasneh[16] found that *Artemisia judaica* from Jordan has antimicrobial activity. He mentioned that water-ether extract had high activity against *K. aerogenes*, *P. aeruginosa* and *S. aureus*

The aims of this study were to test the activity of the plant extracts of twelve species belong to six genera of medicinal plants (*Achillea*, *Salvia*, *Convolvulus*, *Plantago*, *Anthemis* and *Artemisia*) that grown in Jordan on inhibiting the growth of *Candida albicans* and also, study the environmental affects on the activity of some medicinal plants.

MATERIALS AND METHODS

Plant material: Plant materials of twelve species belong to the following genera: *Achillea* sp. *(A. fragrantissima, A. biebersteinii and A. santolina), Salvia* sp. *(S. dominica, S. officinalis and S. spinosa), Convolvulus* sp. *(C. althaeoides and C. arvensis), Plantago* sp. *(P. lanceolata and P. major), Anthemis pseudocotula* and *Artemisia herba-alba* were tested in this study. All plants were collected from Jordan habitats. Scientific, family and english names, used part for extraction and common medicinal uses for these plants are summarized in Table 1.

Seeds of two species (*P. lanceolata* and *C. arvensis*) were obtained from France (B & T World Seeds, Paguignan, France). The seeds were cultured under Jordan environment and compared with the same species that collected from Jordan to investigate the environmental effect on their antimicrobial activity.

Preparation of extracts: The plant materials were dried in shade at room temperature and ground by using a blender. Two hundred and fifty gram of plant powder was soaked in 1.25-1.5 L of 95% ethanol for 5 days at room temperature. The mixture was mixed daily for regular infusion. After a five-day period, the extract was filtered by using Whatman filter paper No. 1. The filtrate was dried by using a rotary evaporator at 60°C. The dried extract was stored in sterile glass bottles at –20°C until using[31].

Screening of antimicrobial activities: Nutrient agar medium was prepared and autoclaved at 121°C under 15 psi pressures for 30 min. After cooling to about 65°C, 25 mL of the medium poured in petri-dish [90 x 15 mm (diameter x height)]. The plates kept at room temperature for solidification and stored at 4°C until using.

The activity of the plant extracts was tested against *Candida albicans,* which obtained from the hospital of the University of Jordan. Inoculums containing 10^8 cells mL^{-1} were spreaded on the medium. Antimicrobial activity test was then carried out by using the hole-plate diffusion method. Holes were made on the medium by using 6 mm cork borer. The dried plant extracts were dissolved in dimethylsulfoxide (DMSO) to final extract amounts of 200, 150, 100 and 50 mg mL^{-1}. Each hole (diameter 6 mm) in each plate was filled with 50 µL of plant extract.

The inoculated agar plates were incubated at 37°C for 24 h. After the incubation period, the diameter of inhibition zone to each hole was measured in millimeter. The inhibition zone is the area surrounding the hole and there is no growth of the inoculated microorganism. DMSO used as negative control.

Table 1: Scientific and English names, used part for extraction, and the common medicinal uses of plant species used in this study

Scientific name	English name	Used part for extraction	Common medicinal uses	References
Achillea bieberstinii	Yarrow	Aerial parts	Carminative, insect repellant	[33]
Achillea fragrantissima	Lavender cotton	Aerial parts	Carminative, depurative, antispasmodic, anti-diabetic, anti-diuretic, used for stomachic, internal hemorrhage, tumors, infections, severe cough, fever, rheumatic pain.	[6,10,12,34]
Achillea santolina	Santolina milfoils	Aerial parts	Anti-colic, kidney stones, anti-diabetic, tooth pain, dysentery, carminative, insect repellant	[13,18,33,35]
Salvia dominica	Sage	Leaves	Cold, stomach pain, indigestion	[13]
Salvia officinalis	White sage (common sage)	Leaves	Stomach pain, gargle, antiseptic, astringent, antispasmodic, anti-inflammation	[18,22]
Salvia spinosa	Spiny-calyxed sage	Leaves	Anti-stomach disturbances, anti-inflammatory gargle, antiseptic, anti-tussive, anti-hemorrhoids pain, anti-rheumatic, astringent, carminative, hypotensive	[13]
Convolvulus althaeoides	Mallow-leaved, bindweed	Aerial parts	Laxative, wound healing, asthma	[13,33]
Convolvulus arvensis	Field bindweed	Aerial parts	Laxative, wound healing, antispasmodic, anti-hemorrhagic	[13,18,35]
Plantago lanceolata	Ribwort plantain	Aerial parts	For tooth pain, bronchitis, purgatives, wound healing, cough	[13,18]
Plantago major	Greater plantain	Aerial parts	For stomach upset, stomach ache, stomach and intestine inflammation, abscesses, cold, pimples, wounds, dysentery, burns, angina, asthma, fever, tuberculosis, whooping cough, chronic renal inflammation, dermal diseases, bronchitis, purgative	[13,18,34]
Anthemis pseudocotula	Chamomile	Aerial parts	Anti-diabetic, anti-tumor, antifungal, bactericidal	[13]
Artemisia herba-alba	Herba-alba, wormwood, white mugwort	Flowers and leaves	Cough, stomach and intestine pains, anti-pyretic, eye diseases, hair pomades, hypoglycemic, anti-infections, antibacterial	[13,18]

Statistical analysis: The experiment for the antimicrobial activity was conducted and analyzed as a factorial experiment with four replications in a Completely Randomized Design.

RESULTS

***Achillea* sp.:** Effect of plant species, extract amounts and their interaction were highly significant (Table 2). The three *Achillea* sp. (*A. fragrantissima*, *A. biebersteinii* and *A. santolina*) showed different antimicrobial activity in different extract amounts against the tested fungi (Table 3).

A. fragrantissima inhibited the growth of *C. albicans* at two extract amount (200 and 150 mg mL^{-1}) and gave inhibition zone of 11.25 and 9.75 mm, respectively. The inhibition zones that produced by *A. biebersteinii* were 13.25, 10.50 and 8 mm at extract amounts 200, 150 and 100 mg mL^{-1}, respectively. The extract of *A. santolina* inhibited the growth of *C. albicans* at all used extract amounts (200, 150, 100 and 50 mg mL^{-1}); the inhibition zones were 12.25, 11, 9.75 and 8.50 mm, respectively.

Table 2: Mean squares and degree of freedom (df) from analysis of variance for the antimicrobial activity of three *Achillea* sp. at four extract amounts against *C. albicans*

Source of variation	df	Mean square
Plant (P)	2	18.1458**
Extract amount (E)	3	71.7430**
P*E	6	3.2847**
Error	36	5.7500
Total	47	

Table 3: Antimicrobial activity (represented by inhibition zone/ mm) of the three *Achillea* sp. at four extracts amounts against *C. albicans*

	Extract amounts (mg mL^{-1})			
Plant species	200	150	100	50
A. fragrantissima	11.25	9.75	0	0
A. biebersteinii	13.25	10.50	8	0
A. santolina	12.25	11.00	9.75	8.50

Table 4: Mean squares and degree of freedom (df) from analysis of variance for the antimicrobial activity of three *Salvia* sp. at four extract amounts against *C. Albicans*

Source of variation	df	Mean square
Plant (P)	2	102.5833**
Extract amount (E)	3	8.0556**
P*E	6	2.0556**
Error	36	0.1389
Total	47	

Table 5: Antimicrobial activity (represented by inhibition zone/ mm) of the three *Salvia* sp. at four extracts amounts against *C. albicans*

	Extract amounts mg mL^{-1}			
Plant species	200	150	100	50
S. officinalis	11.25	10.00	9	8.25
S. dominica	12.25	11.25	10.5	9
S. spinosa	0	0	0	0

The highest inhibition zone (13.25 mm) was produced by *A. biebersteinii* extract at 200 mg mL^{-1}.

***Salvia* sp.:** The effect of *Salvia* plants, extract amounts and their interaction were highly significant (Table 4). The extract of *S. officinalis* inhibited the growth of *C. albicans* and produced inhibition zones of (11.25, 10, 9 and 8.25 mm) at all used extract amounts

Table 6: Mean squares and degree of freedom (df) from analysis of variance for the antimicrobial activity of two *Convolvulus* sp. at four extract amounts against *C. Albicans*

Source of variation	df	Mean square
Plant (P)	1	12.5000**
Extract amount (E)	3	4.9167**
P*E	3	4.9167**
Error	24	0.0625
Total	31	

Table 7: Antimicrobial activity (represented by inhibition zone mm) of the two *Convolvulus* sp. at four extracts amounts against *C. albicans*

	Extract amounts mg mL^{-1}			
Plant species	200	150	100	50
C. althaeoides	0	0	0	0
C. arvensis	9.25	7.25	0	0

Table 8: Mean squares and degree of freedom (df) from analysis of variance for the antimicrobial activity of two *Plantago lanceolata* (from Jordan and France) at four extract amounts against *C. Albicans*

Source of variation	df	Mean square
Plant (P)	1	7.0312**
Extract amount (E)	3	7.0312**
P*E	3	7.0312**
Error	24	0.0312
Total	31	

Table 9: Antimicrobial activity (represented by inhibition zone/ mm) of two *Plantago lanceolata* (from Jordan and introduced from France) at four extract amounts against *C. albicans*

	Extract amounts mg mL^{-1}			
Plant species	200	150	100	50
P. lanceolata (Jordan)	0	0	0	0
P. lanceolata (France)	9.75	0	0	0

(200, 150, 100 and 50 mg mL^{-1}), respectively (Table 5). For *S. dominica,* the inhibition zone diameters were 12.25, 11.25, 10.50 and 9 mm with the extract amounts 200, 150, 100 and 50 mg mL^{-1}, respectively. *S. spinosa* showed no activity against *C. albicans*. The highest inhibition was produced by *S. dominica* at 200 mg mL^{-1}.

***Convolvulus* sp.:** *C. althaeoides* and *C. arvensis* were used to study their activity against *C. albicans*. The effects of plant species, extract amounts and their interaction were highly significant (Table 6). Extract of *C. althaeoides* showed no activity against *C. albicans* growth (Table 7), while *C. arvensis* inhibited the fungi growth at two extract amounts (200 and 150 mg mL^{-1}), which produced inhibition zone of 9.25 and 7.25 mm, respectively.

***Plantago* sp.:** *P. lanceolata* and *P. major* that collected from Jordan showed no activity against *C. albicans* at all used extract amounts. There was a significant difference between the antimicrobial activity of *P. lanceolata,* which introduced from France and the same species that collected from Jordan (Table 8). *P. lanceolata* which introduced from France inhibited *C. albicans* growth (9.75 mm) at 200 mg mL^{-1} only while the collected from Jordan shown no activity at any tested extract amount (Table 9).

Table 10: Mean squares and degree of freedom (df) from analysis of variance for the antimicrobial activity of *A. pseudocotula* and *A. herba-alba* at four extract amounts against *C. Albicans*

Source of variation	df	Mean square
Plant (P)	1	8.00**
Extract amounts (E)	3	80.54**
P*E	3	6.75**
Error	24	0.17
Total	31	

Table 11: Antimicrobial activity (represented by inhibition zone mm) of *Anthemis pseudocotula* and *Artemisia herba-alba* at four extract amounts against *C. albicans*

	Extract amounts mg mL^{-1}			
Plant species	200	150	100	50
A. pseudocotula	13.2	10.5	0	0
A. herba-alba	13.2	10.7	9.7	0

***Anthemis pseudocotula* and *Artemisia herba-alba*:** *A. pseudocotula* inhibited the growth of *C. albicans* at two extract amounts (Table 11). The inhibition zones were 13.2 and 10.5 mm at extract amounts 200 and 150 mg mL^{-1}, respectively. While the extract of *A. herba-alba* showed antimicrobial activity against *C. albicans* at three extract amounts (200, 150, 100 mg mL^{-1}) and the inhibition zones were 13.2, 10.7 and 9.7 mm, respectively. The differences between the plants, the used extract amounts and their interactions were highly significant (Table 10).

DISCUSSION

Among *Achillea* species, *A. santolina* was very active against *C. albicans*. It inhibited the *Candida* growth at the four extract amounts. *A. biebersteinii* was also active at three extract amounts (200, 150 and 100 mg mL^{-1}). It seemed that *C. albicans* is sensitive to *A. santolina* at all extract amounts and may be even below 50 mg mL^{-1}, while it was sensitive to the other *Achillea* species at high concentrations (200 and 150 mg mL^{-1}).

The collected plants of *P. lanceolata* from Jordan and France showed different response in their antimicrobial activity. Jordanian *P. lanceolata* showed no activity while French *P. lanceolata* revealed activity on the growth of *C. albicans* at extract amount (200 mg mL^{-1}) only. This variation in quality or composition of the same plant species could be due to differences in the environmental conditions and genetic variations.

The extract of *S. officinalis and Salvia dominica* inhibited the growth of *C. albicans* at all four tested extract amounts. This result indicated that *C. albicans* was sensitive to the extracts of these two species and was

resistant to extract of *Salvia spinosa*, which produced no antimicrobial activity at any extract amount.

Skoula *et al.*[32] worked on *Salvia fruticosa* that collected from different geographic locations in the island of Crete. They found that the essential oils of these plants gave different response when all plants cultured under the same condition for three years. They suggested that this result was due to variation in quality and composition of plants and this might depends more on the genetic background of the plants and less on climate variation.

C. albicans was resistance to the extract of *Convolvulus althaeoides*; it was affected by extract of *Convolvulus arvensis* at extract amounts (200 and 150 mg mL^{-1}). This could be due to the genetic variations between the two species or higher concentrations of extract need to be used.

The lowest extract amount (50 mg mL^{-1}) of *Anthemis pseudocotula* and *Artemisia. heba-alba* showed no antimicrobial activity against the growth of *C. albicans*. This means that the extract of these two genera need to be applied at high concentrations.

Present results indicated that the plant species used in this study could have an antimicrobial agent that caused the activity of these medicinal plants against *C. albicans*. Also, they could have the same agent but in different concentrations that causes high variations in their antimicrobial activity.

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