

Antimicrobial Activity of some Medicinal Plants against Different *Candida* Species

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ABSTRACT

The anticandidal activity of nineteen Jordanian plant extracts (*Capparis spinosa* L., *Artemisia herba-alba* Asso, *Echinops polyceras* Boiss., *Gundelia tournefortii* L., *Varthemia iphionoides* Boiss. & Blanche, *Eruca sativa* Mill., *Euphorbia macroclada* L., *Hypericum trequetrifolium* Turra, *Achillea santolina* L., *Mentha longifolia* Host, *Origanum syriacum* L., *Phlomis brachydo* (Boiss.) Zohary, *Teucrium polium* L., *Anagyris foetida* L., *Trigonella foenum-graecum* L., *Thea sinensis* L., *Hibiscus sabdariffa* L., *Lepidium sativum* L., *Pimpinella anisum* L.) were tested against *Candida albicans* ATCC 10231, *Candida glabrata* ATCC 1615 and *Candida krusei* ATCC 6258) in addition to some other clinical isolates of these species. Fluconazole was used as a reference standard to determine the sensitivity of the strains. The minimum inhibitory concentration ranged from 3.1 to 25 mg ml⁻¹. Most of the extracts exhibited anticandidal effect. Although the *in vitro* obtained findings are promising, the extracts need further studies to be standardized for *in vivo* therapeutic applications.

Keywords: Medicinal Plants, *Candida* spp., Antifungal Agents.

INTRODUCTION

The spread of multidrug-resistant strains of *Candida* and the reduced number of drugs available makes it necessary to search for new sources of antifungals and compounds that inhibit these resistance mechanisms. This has led to a search for therapeutic alternatives, particularly among medicinal plants and compounds isolated from them used for their empirically antifungal properties.

In the past few decades, a worldwide increase in the incidence of fungal infections has been observed as well as a rise in the resistance of some species of fungus to different fungicidal used in medicinal practice¹.

The incidence of systemic candidiasis infections caused by pathogenic yeasts especially *Candida* spp. has increased considerably in critically ill patients².

This is mainly due to the increased number of immunocompromised-patients as a result of new and more aggressive therapies in the treatment of cancer and tumor. Other factors may include the increase of

immunosuppressive drugs and the number of organ transplant recipients³.

Studies of Acquired Immune Deficiency Syndrome (AIDS) all over the world show that about 58–81% of all patients contract a fungal infection especially candidiasis⁴. On top of that, about 10–20% of the patients died as a direct consequence of fungal infections⁵.

These facts coupled with the resistance to antifungals and with the toxicity during prolonged treatment using several antifungal drugs have been reasons for an extended search for newer drugs to treat candidiasis.

Although there are several synthetic and natural product-based drugs available for treating candidiasis, they are not consistently effective against pathogenic yeast infections⁶. Furthermore, the development of resistance in fungi against most of the drugs has been reported⁷.

Hence, there is a great demand for novel antifungals belonging to a wide range of structural classes, selectively acting on new targets with fewer side effects. One approach might be the testing of plants traditionally

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used for their antifungal activities as potential sources for drug development. Medicinal plants are not only important to the millions of people for whom traditional medicine is the only opportunity for health care and to those who use plants for various purposes in their daily lives, but also as a source of new pharmaceuticals. Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the matchless availability of chemical diversity⁸.

Medicinal plants constitute an important source of bioactive compounds and the use of medicinal plants in the treatment of infections is well known. Plants produce many types of secondary metabolites; many of them with antifungal activities. Examples of these compounds

include flavonoids, phenolics and phenolic glycosides, unsaturated lactones, sulphur compounds, saponins, cyanogenic glycosides and glucosinolates⁹.

In many cases, these substances serve as plant defense mechanisms against predation by microorganisms, insects and herbivores.

In this study, nineteen Jordanian plants, known to have antimicrobial activity in folk medicine were screened for their possible antifungal effect¹⁰⁻¹⁴. Some of them are edible and considered safe. In general, these plants are used in folk medicine in the treatment of skin diseases, gastrointestinal tract diseases and respiratory problems. The plants used in this study and their uses are listed in Table (1).

Table 1. The ethnomedicinal plants and their uses in folklore medicine used in this study .

	Scientific Name (voucher specimen)	% Yield	Part used	Claimed Usage
1	<i>Achillea santolina</i> L. (AS-M99) (Labiatae)	7.9	Aerial Parts	Carminative, Depurative, Stomachaches, antispasmodic and diabetes
2	<i>Anagyris foetida</i> L. (Al-abd. 99-2) (Leguminosae)	8.6	Leaves & fruits	laxative, pectoral, purgative, vermifuge
3	<i>Artemisia herba-alba</i> Asso. (Abadi 00-8) (Compositae)	4.5	Foliage	Antidiabetic, Antispasmodic, pectoral, antiarthritis
4	<i>Capparis spinosa</i> L. (Abadi 99-20) (Capparidaceae)	6	Roots	Rheumatic pain Purgative and anthelmentic
5	<i>Echinops polyceras</i> Boiss. (Al-abd. 99-3) (Compositae)	9.1	Whole plant	Wounds and warts
6	<i>Eruca sativa</i> Mill. (ES-M99) (Cruciferae)	11.2	Fruits	Aphrodisiac Antispasmodic and for renal colic
7	<i>Euphorbia macroclada</i> L. (Al-abd. 98-11) (Euphorbiaceae)	0.8	Latex	Urticaria, warts

	Scientific Name (voucher specimen)	% Yield	Part used	Claimed Usage
8	<i>Euphorbia macroclada</i> L. (Al-abd. 98-1) (Euphorbiaceae)	6.6	whole plant	Urticaria, warts
9	<i>Gundelia tournefortii</i> L (Abbadi 00-24) (Compositae)	6.7	Whole plant	Edible like artichoke, antioxidant, treatment of vitiligo, diuretic
10	<i>Hypericum trequetrifolium</i> Turra (Abbadi 99-23) (Gittiferae)	5.5	Arial parts	Toxic Antidepressant in cases of mania
11	<i>Mentha longifolia</i> Host (ML-99) (Labiatae)	10.6	Leaves	Constipation, fever, common cold, general weakness
12	<i>Origanum syriacum</i> L. (Majorana syriaca (L.) Raf. (Abbadi 00-19) (Labiatae)	9.6	Leaves	Carminative, pectoral, antitussive, aperative, antistomach ache, Carminative.
13	<i>Phlomis brachydon</i> (Boiss.) Zohary (Al-Abd. 99-4) (Labiatae)	3.6	Whole plant	Stomach and intestine pain
14	<i>Teucrium polium</i> L. (Abbadi 99-5) (Labiatae)	11.9	Aerial parts	Spasm, flatulence, diabetes and kidney stones
15	<i>Trigonella foenum-graecum</i> L. (TF-M99) (Leguminosae)	6.3	Seeds	Diabetes, sexual impotence, intestinal pain, infant abdominal pain, skin diseases
16	<i>Thea sinensis</i> L. (TS-m99) (Theaceae)	5.6	leaves	Drink, externally anti-inflammatory
17	<i>Hibiscus sabdariffa</i> L. (Abbadi 00-180) (Malvaceae)	6.4	Calyx	Drink, antihypertensive

	Scientific Name (voucher specimen)	% Yield	Part used	Claimed Usage
18	<i>Lepidium sativum</i> L. (LS-M99) (Umbelliferae)	3.8	Seeds. Fresh plant	General tonic
19	<i>Pimpinella anisum</i> L. (PA-M99) (Umbelliferae)	9.9	Fruit	Antiflatulance and antispasmodic
20	<i>Varthemia iphionoides</i> Boiss. & Blanche (Val-M99) (Compositae)	8.8	Leaves and Stems	Women sterility, female fertilization, eye infection, antispasmodic Anti-inflammatory, diabetes. Women delivery

MATERIALS AND METHODS

Preparation of Plant Extracts

Air dried and finely powdered plant materials were extracted in a Soxhlet with two liters of methanol for 4 hrs, except for *Capparis spinosa*, which was extracted for 10 hrs.

Solvents were then evaporated under reduced pressure and the extracts were conserved in tightly sealed glass vials. *Euphorbia macroclada* latex was obtained by cutting and squeezing the stem of the plant and examined directly¹⁰.

Yeast Cultures

Clinical isolates of pathogenic yeast used in this study (*Candida albicans*, *Candida glabrata* and *Candida krusei*) were obtained from hospitalized patients at the Jordan University Hospital and were identified by biochemical tests. Standard strains of *Candida albicans* ATCC 10231, *Candida glabrata* ATCC 1615, and *Candida krusei* ATCC 6258 were used as reference strains. Cultures were grown and maintained on Sabouraud glucose agar slopes at 37 °C for 24–48 hrs.

Agar Diffusion Susceptibility Test

Antifungal activity tests were performed using agar diffusion method¹⁵. Inocula of the test *Candida* spp. were standardized by making water suspensions from the 24 hr cultures and adjusted to 1×10^8 cfu/ml as per Mcfarland standard. One milliliter of yeast cell suspension was

incorporated into 15 ml molten Sabouraud glucose agar maintained at 45 °C. The agar was then poured into sterile Petri plates and allowed to solidify at room temperature. Fluconazole and dried methanolic extracts of medicinal herbs were dissolved in absolute ethanol. Discs (8 mm) (Whatman, Kent, UK) were soaked in these solutions to give final contents of 200 µg of fluconazole and 4000 µg of medicinal herbs. The prepared discs, plus blank controls, were dried then applied to the plates, which were placed in an incubator within 15 min, and incubated at 35°C for 48 hrs. Antifungal activity was indicated by a clear zone of growth inhibition formed around the disc. The experiments were carried out in triplicate.

Minimum Inhibitory Concentration (MIC)

The MIC was carried out according to the NCCLS guidelines (16). In brief, 100 µl of broth were added to each well of a 96-well microtitration plate. One hundred microlitres of the plant extract were added to well (A) and serially diluted. Two-fold serial dilutions (B_H) were prepared from extract (A), 100 µl from the last well (H) were discarded. In case of organic solvent extracts, initial concentration of 25 µl of extract was added to 175 µl broth and serially diluted. Triplicate were prepared for each extract. All the wells were then filled with 100 µl of stock yeast culture. Fluconazole was used as a reference for this experiment. The following control wells were parallel prepared: wells contain only broth, other

wells contain *Candida* strain without extract, and serial dilutions of Fluconazole with the fungi at the recommended inhibitory concentrations. The plates were then read at 630 nm, covered with sterile parafilm, and incubated at 33 °C overnight. Absorbance was reread at the same wavelength.

RESULTS AND DISCUSSION

Plants used in this study are mentioned in Table (1). The *Candida* spp. used in this study were chosen on the basis of their pathogenic role to humans. According to findings from the National Nosocomial Infection Surveillance System (NNISS), USA, 61% of reported nosocomial fungal infections were due to *Candida*

albicans, followed by other *Candida* spp.¹⁷. Although *Candida albicans* is counted as one of the microbial flora in humans, it may convert to induce oral thrush or systematic infection.

The results of the antifungal screening of nineteen medicinal plants are presented in Tables (2 and 3). Among the tested plants, the methanolic extract of each of *Artemisia herba-alba* Asso., *Echinops polyceras* Boiss., *Mentha longifolia* Host, *Phlomis brachydon* (Boiss.) Zohary, *Thea sinensis* L., and *Varthemia iphionoides* Boiss. & Blanche revealed the highest activity against *Candida albicans* with an MIC value of 6.3 mg ml⁻¹. These plants were also active against clinical *Candida* isolates with higher MIC value of 25.0 mg ml⁻¹.

Table 2. Growth inhibition of methanolic extracts against clinical *Candida* isolates

	Scientific Name (voucher specimen)	Family Name Labiataeae	Microorganisms tested					
			CA	CG	CK	CAC	CGc	CKc
1	<i>Achillea santolina</i> L. (AS-M99)	Labiataeae	-	-	-	-	-	-
2	<i>Anagyris foetida</i> L. (Al-abd. 99-2)	Leguminosae	+	+	+	-	-	-
3	<i>Artemisia herba-alba</i> Asso. (Abbadi 00-8)	Compositae	+	+	+	-	-	-
4	<i>Capparis spinosa</i> L. (Abbadi 99-20)	Capparidaceae	+	+	+	-	-	-
5	<i>Echinops polyceras</i> Boiss. (Al-abd. 99-3)	Compositae	+	+	+	+	+	+
6	<i>Eruca sativa</i> Mill. (ES-M99)	Cruciferae	-	-	-	-	-	-
7	<i>Euphorbia macroclada</i> L. (Al-abd. 98-11)	Euphorbiaceae	+	+	+	+	+	+
8	<i>Euphorbia macroclada</i> L. (Al-	Euphorbiaceae	+	+	+	+	+	+

	Scientific Name (voucher specimen)	Family Name Labiataeae	Microorganisms tested					
			CA	CG	CK	CAC	CGc	CKc
	abd. 98-1)							
9	<i>Gundelia tournefortii</i> L (Abbadi 00-24)	Compositae	-	-	-	-	-	-
10	<i>Hypericum trequetriifolium</i> Turra (Abbadi 99-23)	Gittiferae	+	+	+	-	-	-
11	<i>Mentha longifolia</i> Host (ML-99)	Labiataeae	+	+	+	+	+	+
12	<i>Origanum syriacum</i> L. (Majorana syriaca (L.) Raf. (Abbadi 00-19)	Labiataeae	+	+	+	+	+	+
13	<i>Phlomis brachydon</i> (Boiss.) Zohary (Al-Abd. 99-4)	Labiataeae	+	+	+	-	-	-
14	<i>Teucrium polium</i> L. (Abbadi 99-5)	Labiataeae	-	-	-	-	-	-
15	<i>Trigonella foenum- graecum</i> L. (TF-M99)	Leguminosae	+	-	+	-	-	-
16	<i>Thea sinensis</i> L. (TS-m99)	Theaceae	+	+	+	+	+	+
17	<i>Hibiscus sabdariffa</i> L. (Abbadi 00-180)	Malvaceae	-	-	+	-	-	-
18	<i>Lepidium sativum</i> L. (LS-M99)	Umbelliferae	+	-	-	-	-	-

	Scientific Name (voucher specimen)	Family Name Labiatae	Microorganisms tested					
			CA	CG	CK	CAC	CGc	CKc
19	<i>Pimpinella anisum</i> L. (PA-M99)	Umbelliferae	+	+	+	+	+	+
20	<i>Varthemia iphionoides</i> Boiss. & Blanche (VaI-M99)	Compositae	+	+	-	-	-	-

CA: *Candida albicans*; CG: *Candida glabrata*; CK: *Candida krusei*; CAC: *Candida albicans* (Clinical isolate); CGc: *Candida glabrata* (Clinical isolate); CKc: *Candida krusei* (Clinical isolate);

-: no inhibition zone formed; +: inhibition zone formed

The clinical *Candida* isolates were found to be resistant to most plant extracts screened except for the six above mentioned plants. Amongst the reference *Candida* spp, *Candida glabrata* was less susceptible to most plant extracts. The *Echinops polyceras* Boiss. and *Phlomis brachydon* (Boiss.) Zohary were active against *Candida krusei* with an MIC value of 3.1 mg ml⁻¹. The extracts of *Teucrium polium*

L., *Gundelia tournefortii* L, and *Eruca sativa* Mill. were found ineffective against all reference and clinical isolates (Tables 2 and 3). *Hibiscus sabdariffa* inhibited the growth of *Candida krusei* with an MIC value of 6.3 mg ml⁻¹ however, it did not have any effect on the other *Candida* species or the clinical isolates.

Table 3. The minimum Inhibitory Concentrations (MIC) values (mgml⁻¹) of methanol extracts of some plants against *Candida* spp. Tested using the microdilution method

	Plant	MIC					
		CA	CG	CK	CAC	CGc	CKc
1	<i>Anagyris foetida</i> L. (Leguminosae)	12.5± 1.8	25.0 ± 2.0	12.5±3.1	*NT	*NT	*NT
2	<i>Artemisia herba-alba</i> Asso. Compositae	6.3 ± 0.8	25.0 ± 5.5	12.5±4.5	*NT	*NT	*NT
3	<i>Capparis spinosa</i> L. Capparidaceae	12.5± 2.5	12.5± 1.9	12.5 ± 4.1	*NT	*NT	*NT

	Plant	MIC					
		CA	CG	CK	CAc	CGc	CKc
4	<i>Echinops polyceras</i> Boiss. Compositae	6.3 ± 3.6	12.5± 4.8	3.1 ± 5.6	*NT	*NT	*NT
5	<i>Euphorbia macroclada</i> L. (Euphorbiaceae)	12.5 ± 1.8	12.5± 5.3	12.5±2.8	25±2.9	25±3.1	25±3.4
6	<i>Euphorbia macroclada</i> L. (Euphorbiaceae)	12.5 ± 9.6	12.5± 2.5	12.5±3.9	25±8.5	25±4.2	25±3.5
7	<i>Hypericum trequetrifolium</i> Turra (Gittiferae)	12.5± 2.8	25±6.1	12.5±8.1	*NT	*NT	*NT
8	<i>Mentha longifolia</i> Host (Labiatae)	6.3 ± 3.8	12.5 ± 4.9	12.5 ± 6.1	25.0 ± 5.9	25.0 ± 4.3	25±8.7
9	<i>Origanum syriacum</i> L. (Majorana syriaca (L.) Raf (Labiatae)	6.3 ±3.5	12.5 ± 4.9	12.5 ± 2.9	25 ± 7.3	25.0 ± 2.8	25.0 ±1.9
10	<i>Phlomis brachydon</i> (Boiss.) Zohary (Labiatae)	6.3 ± 4.2	12.5 ± 1.1	3.1 ± 4.1	*NT	*NT	*NT
11	<i>Trigonella foenum-graecum</i> L. (Leguminosae)	12.5 ± 3.4	*NT	12.5 ± 5.6	*NT	*NT	*NT
12	<i>Thea sinensis</i> L. (Theaceae)	6.3 ± 9.6	12.5 ± 3.9	12.5 ± 6.1	25.0 ± 7.2	25.0 ±3.2	25.0 ± 2.9
13	<i>Hibiscus sabdariffa</i> L. (Malvaceae)	*NT	*NT	25.0 ± 5.5	*NT	*NT	*NT
14	<i>Lepidium sativum</i> L. (Umbelliferae)	25.0 ±9.4	*NT	*NT	*NT	*NT	*NT
15	<i>Pimpinella anisum</i> L. (Umbelliferae)	12.5 ± 7.2	12.5 ± 5.5	12.5 ± 4.2	25.0 ± 3.6	25.0 ±4.6	25±7.1

	Plant	MIC					
		CA	CG	CK	CAc	CGc	CKc
16	<i>Varthemia iphionoides</i> Boiss. & Blanche (Compositae)	6.3 ± 2.9	*NT	*NT	*NT	*NT	*NT
	Fluconazole**	30	40	35	≥65	≥65	≥65

*NT, extract not tested.

**Fluconazole was used as standard for antifungal activity its minimum inhibitory concentration is in (µg/ml)

CA: *Candida albicans*; CG: *Candida glabrata*; CK: *Candida krusei*; CAc: *Candida albicans* (Clinical isolate); CGc: *Candida glabrata* (Clinical isolate); CKc: *Candida krusei* (Clinical isolate);

-: no inhibition zone formed; +: inhibition zone formed

The activity of extracts against the reference strains was more than that revealed on clinical isolates. Such a variation in the activities of extracts, especially of those prepared from plants of the same families, might be due to their chemical structural differences and/or to the mechanism of their active substances (Tables 2 and 3).

Most of the tested plants were active against *Candida albicans*. Eight plants (*Achillea santolina* L., *Eruca sativa* Mill., *Trigonella foenum-graecum* L., *Hibiscus sabdariffa* L., *Lepidium sativum* L., *Varthemia iphionoides*, *Gundelia tournefortii* L and *Teucrium polium* L.) were inactive against *Candida glabrata* indicating that this strain is more resistant. Two extracts (*Gundelia tournefortii* L and *Teucrium polium* L.) were inactive against all tested strains. Differences in the anticandidal activity are obviously related to differences in their contents of active compounds. This might be related to some variation in their active compounds.

It is noteworthy to mention that plants belonging to the same family sometimes exhibited comparable anticandidal activity since these plants are expected to possess similar active constituents (e.g. *Mentha longifolia* Host, *Origanum syriacum* L. *Phlomis brachydon* of

Family Labiateae) however, in other cases plants of the same family exhibited different effects (e.g. *Lepidium sativum* L., and *Pimpinella anisum* L. of the Family Umbelliferae) indicating that there might be some constituents found in one member of the family but not in the other.

The present work has shown that most of the studied plants are potentially a good source of anticandidal agents and demonstrates the importance of such plants in medicine and in assisting primary health care.

CONCLUSION

Overall, the results suggest that among the reference strains *Candida albicans* is the most susceptible fungal strain and the most resistant was *Candida glabrata*. Candidal clinical isolates were generally resistant to most plant extracts used.

Some crude extracts prepared from plants commonly grown in Jordan or commonly used by Jordanians were found to exert, *in-vitro* some antifungal effect. Work is currently being undertaken to isolate the active compound(s) by bioassay-guided fractionation from the species that showed high inhibitory activity during screening.

REFERENCES

- (1) Reimann S. and Deising H. B. Inhibition of efflux transporter-mediated fungicide resistance in *Pyrenophora tritici-repentis* by a derivative of 4-hydroxyflavone and enhancement of fungicide activity applied and environmental microbiology, 2005, 6: 3269-3275.
- (2) Meon M., Marchetti O. and Calandra T. Bench-to bedside review: Candida infections in the intensive care unit. *Critical care* 2008,12:204
- (3) Richardson M.D. Changing patterns and trends in systemic fungal infections, *Journal of antimicrobial chemotherapy* 2005 ,56 Suppl S1:i5-ii1.
- (4) Jabra-Rizk M. A., Ferreira S. M. S., Sabet M., Falkler W. A., Merz W. G. and Meiller T. F. Recovery of *Candida dubliniensis* and Other Yeasts from Human Immunodeficiency Virus-Associated Periodontal Lesions. *Journal of Clinical Microbiology*,2001,39: 4520–4522
- (5) Drouhent E. & Dupont B. Fluconazole for the treatment of fungal diseases in immunosuppressed patients. *Annals New York Academy of Science*, 1989, 544: 564–570.
- (6) Darah I., Jain K., Suraya S., Lim S. H., Hazarinal N. and Adnalizawati A. Siti Noor Screening For Antiyeast Activities From Selected Medicinal Plants. *Journal of Tropical Forest Science* ,2006, 18(4): 231–235 .
- (7) Sanglard D. Resistance of human fungal pathogens to antifungal drugs. *Current Opinion in Microbiology*, 2002, 5: 379–385 .
- (8) José Abad M. J., Ansuategui M., and Paulina Bermejo Active antifungal substances from natural sources ARKIVOC 2007 (vii) 116-145
- (9) Cowan M. M. Plants products as antimicrobial agents. *Clinical Microbiology Reviews*, 1999, 12: 564–582.
- (10) Darwish, RM, Aburjai T, Effect of ethnomedicinal plants used in folklore medicine in Jordan as antibiotic resistant inhibitors on *Escherichia coli* BMC Complement Altern Med. 2010, 28:10:9.
- (11) Aburjai T, Darwish RM, Al-Khalil S, Mahafza A, Al-Abbadi A: Screening of antibiotic resistant inhibitors from local plant materials against two different strains of *Pseudomonas aeruginosa*. *Journal of Ethnopharmacology* 2001, 76: 39-44.
- (12) Darwish, RM, Aburjai T, Al-Khalil S, Mahafza A, Al-Abbadi A: Screening of antibiotic resistant inhibitors from local plant materials against two different strains of *Staphylococcus aureus*. *Journal of Ethnopharmacology* 2002, 79:359-364.
- (13) Al-Khalil S: A Survey of plants used in Jordanian traditional medicine. *International Journal of Pharmacognosy* 1995, 33:317-323.
- (14) Talal Aburjai , Mohammad Hudaib, Rabab Tayyem, Mohammed Yousef, Maher Qishawi : Ethnopharmacological survey of medicinal herbs in Jordan, the Ajloun Heights region . *Journal of Ethnopharmacology* ,2007, 110:294–304
- (15) National Committee for Clinical Laboratory Standards. (1993). Performance Standards for Antimicrobial Disk Susceptibility Tests— Fifth Edition: Approved Standard M2–A5. NCCLS, Villanova, PA.
- (16) National Committee for Clinical Laboratory Standards. (1992). Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts: Proposed Guideline M27–P. NCCLS, Villanova, PA.
- (17) Walsh JJ. Invasive fungal infections: problems and challenges for developing new antifungal compounds. In: Sutcliffe JA, Georgapapadakov NH, eds., Merging Targets in Antibacterial and Antifungal Chemotherapy. New York, Chapman and Hall, 1992; 349-373.
- (18) Motsei M. L., Lindsey K. L., Van Staden J. & Jager A. K. Screening of traditionally used South African plants for antifungal activity against *Candida albicans*. *Journal of Ethnopharmacology*, 2003, 86: 235–241.

(Candida spp.)

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(ATCC 10231)

(ATCC 6258) (ATCC 1615)

(25.000 – 3.125 mg/ml) (MIC)

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