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# *In vitro* effect of some plant extracts on mycelial growth of *Candida* species

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## ABSTRACT

Crude extracts of the two plants (*Lepidium sativum* and *Saltera sarcocolla*) were evaluated *in vitro* as antifungal on the mycelial growth (dry and fresh weights) of *Candida* species. Two concentrations (2 and 4%) were used from the tested plant extracts. They exhibited inhibitory effect which varied not only with the plant extracts but also with the fungal species. Both concentrations of the two plant extracts significantly affected all the tested fungal species when compared to control. *S. sarcocolla* extract was more effective than *L. sativum* extract on inhibitory mycelial growth of the four introduced fungi or domestic isolates of *Candida* species. The extracts of two plant species were more effective on the mycelial growth of *Candida albicans* as compared to the other tested *Candida* species or control. In addition, employing high concentration of the tested extracts gave significantly higher activity in reducing the mycelial growth than using low concentration.

## INTRODUCTION

It is well known that several plants harbour exhibit a great variety of medications which continue to be a stable source for antimicrobial agents (Al-Rawi, 1964), and the use of natural substance for control of pathogenic fungi due to protection ecological and health (El-Safwani and Aly, 2003). The medicinal and aromatic plants also documented as antifungal agents (Sivropoulou *et al*., 1995 and Heweidy *et al*., 1997). In a previous study, Arora *et al*., (1999) reported that the medicinal value of two plant species, *Rosemarinus officinalis* and *Eugenia aromatica*. In this study, two other plant species were similarly investigated for their medicinal value. These were garden cress, *L. sativum* and *S. sarcocolla*. The former was reported to be of a great value in the treatment of asthma, cough, and bleeding piles. Leaves of this plant are used in the scorbutic diseases, hepatic disorders, and

anti-fertility drug (Kamboj and Dhawan, 1982; Saksena and Tripathi, 1987 and Cowan, 1999). *L. sativum* was found to be an excellent source of raw material for valuable medicinal compounds such as benzylisothiocynate, benzyl cyanide and an alkaloid (Lepidine), which possesses anti-fertility activity (Pande *et al*., 1999), chemoprotective effects on the 1Q-induced genotoxic effect and colonic preneoplastic lesions (Fekadukassie *et al*., 2002) and antioxidant activity that might increase resistance against stress (Kyeong-Jun Lee *et al*., 2005).

*S. sarcocolla* (Penaeaceae) has long been reported for its medicinal value (Felter and Lioyd, 1998). It was reported to contains sarcocolla (Sarcocollin), a white gummy material which was used to heal wounds, check otorrhoea and as an application to scrofulous enlargements and chronic articular inflammations. Plants belonging to families related to penaeaceae were also reported to contain anti-feedant, anti-microbial agents, phytotoxic acaricides, insecticides and nematicides (Dgweno Midiwo *et al*., 2002).

This study was carried out to evaluate the *in vitro* effect of *Saltera sarcocolla* and *Lepidium sativum* plant extracts on the mycelial growth (dry and fresh weights) of *Candida* species.

## MATERIALS AND METHODS

**Source of fungal species:** Four standard fungal species, namely, *Candida tropicalis, C. parapsilosis, C. albicans* and *C. krusei*, were used in this study. These fungal species causing female genital tract inflammation were obtained from Difico Laboratories, Mi., U.S.A. Three clinical isolates of each fungal species (previously mentioned) were obtained from three patients at Riyadh Armed force hospital, Saudi Arabia. Each fungal species was propagated on sterilized Czapek's agar medium for further studies.

**Plant materials:** Extracts of all parts of two medicinal and aromatic plants (*Lepidium sativum* Linn and *Saltera sarcocolla* (Penaeaceae) were tested for their antifungal activity.

**Preparation of crude extracts:** Samples of 40 g of each *L. sativum* or *S. sarcocolla* were boiled separately in 100 ml sterilized distilled water for 30 min. The extracts were filtered through two layers of sterilized cheese cloth.

The filtrate was centrifuged at 400 rpm for 20 min. The yielded supernatants were sterilized through centered glass (G4) to be used in further studies.

***In vitro* effect of crude extracts of *L. sativum* and *S. sarcocolla* on *Candida* species:** The concentrations (2 and 4%) of each plant were prepared by adding suitable amount of sterilized distilled water to the crude extracts of either *L. sativum* or *S. sarcocolla* (V/V). Two or four ml of each tested concentration was added to Czapek's broth medium in conical flasks before solidification. Inoculation was done with fungal discs, 5 mm in diameter obtained from *Candida* standard species and three clinical isolates of each *Candida* species, 7 days old culture. Three replicates were used for each tested concentration. Another group of Czapek's broth conicals free from plant extracts, inoculated with the fungus species was used as a check treatment. The mean values of the replicates were estimated. Dry and fresh weights were recorded after an incubation period of 7 days at 25±2°C. The obtained data were statistically analyzed, according to Snedecor and Cochran (1967).

## RESULTS AND DISCUSSION

***In vitro* effect of aqueous extracts of *L. sarcocolla* and *L. sativum* on growth of *Candida* species:**

**1. *Candida* standard species:** The antifungal properties of two aqueous plant extracts against three *Candida* standard species were evaluated *in vitro*. Data in Table (1) showed that extract of *S. sarcocolla* significantly reduced growth (fresh weight) of the four tested fungi, at the two tested rates (2 and 4% v/v), when compared to the control, whereas that extract of *L. sativum* gave similar activity except, for both *Candida tropicalis* and *C. albicans* which were not affected at the rate of 2%. In addition**,** the effect of both extracts was comparable against the four tested fungi, especially at higher concentration (4%). In case of lower concentration (2%), all of the tested fungi, in terms of dry weight of mycelial growth, were not affected, except extract of *L. sativum* was not affected with two tested concentrations. Data also showed that *S. sarcocolla* extract was more effective as antifungal agent against *Candida* species than *L. sativum*.

**2. Clinical isolates of the four *Candida* species:** Data in Table (2) showed that the aqueous extracts of both plant species significantly affected both mycelial growth (dry or fresh weights) of *Candida tropicalis* when compared to control, but a minor differences between isolates of the same

Table (1). Effect of *Saltera sarcocolla* and *Lepidium sativum* extracts on the Candida standard species.

| Plant extracts | Rate g/l | Candida standard species | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | *C. tropicalis* | | *C. parapsilosis* | | *C. albicans* | | *C. krusei* | |
| | | Fresh weight (mg) | Dry weight (mg) | Fresh weight (mg) | Dry weight (mg) | Fresh weight (mg) | Dry weight (mg) | Fresh weight (mg) | Dry weight (mg) |
| *Saltera sarcocolla* | 20 | 46.11 | 33.50 | 45.71 | 32.30 | 42.22 | 32.50 | 44.15 | 33.00 |
| | 40 | 42.50 | 30.45 | 42.50 | 31.56 | 39.45 | 30.50 | 43.00 | 32.00 |
| $\overline{X}$ | | 44.30 | 31.97 | 44.10 | 31.93 | 40.85 | 31.50 | 43.57 | 32.50 |
| *Lepidium sativum* | 20 | 48.40 | 35.33 | 46.20 | 34.36 | 48.00 | 33.00 | 46.50 | 36.10 |
| | 40 | 44.24 | 31.50 | 43.50 | 33.55 | 41.30 | 32.70 | 46.20 | 33.10 |
| $\overline{X}$ | | 46.32 | 33.41 | 44.85 | 33.95 | 44.65 | 32.85 | 46.35 | 34.60 |
| Control | 0 | 50.23 | 33.24 | 51.50 | 34.30 | 50.00 | 35.00 | 50.00 | 35.00 |

L.S.D. at 5% between: Extract (a): 2.09; Fungal (b): 3.04; Rate (c): 1.23; a x b x c: 4.05

Table (2). Effect of of *Saltera sarcocolla* and *Lepidium sativum* extracts on three clinical isolates of *Candidia* from patients at one of Riyadh Hospitals.

| Plant extracts | Rate g/l | *Candida* clinical isolates | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *Candida tropicalis* isolates | | | | | | *C. parapsilosis* isolates | | | | | |
| | | (11) | | (12) | | (13) | | (11) | | (12) | | (13) | |
| | | Fresh weight (mg) | Dry weight (mg) | Fresh weight (mg) | Dry weight (mg) | Fresh weight (mg) | Dry weight (mg) | Fresh weight (mg) | Dry weight (mg) | Fresh weight (mg) | Dry weight (mg) | Fresh weight (mg) | Dry weight (mg) |
| *Saltera sarcocolla* | 2 | 46.20 | 30.00 | 46.10 | 29.11 | 46.11 | 29.11 | 45.70 | 32.78 | 44.60 | 32.45 | 45.00 | 32.11 |
| | 4 | 42.00 | 24.11 | 40.22 | 23.10 | 41.00 | 24.25 | 42.41 | 30.40 | 42.11 | 30.00 | 42.10 | 31.27 |
| | $\overline{X}$ | 44.10 | 27.05 | 43.11 | 26.10 | 43.55 | 26.68 | 44.05 | 31.59 | 43.35 | 31.22 | 43.55 | 31.69 |
| *Lepidium sativum* | 2 | 48.45 | 32.12 | 47.00 | 31.12 | 43.99 | 31.90 | 46.00 | 32.53 | 44.00 | 32.22 | 46.98 | 31.00 |
| | 4 | 44.25 | 26.23 | 40.00 | 25.23 | 39.98 | 26.00 | 43.33 | 30.38 | 42.98 | 30.25 | 42.87 | 30.00 |
| | $\overline{X}$ | 46.35 | 29.17 | 43.50 | 25.17 | 41.98 | 28.99 | 44.66 | 31.45 | 44.49 | 31.23 | 44.92 | 30.50 |
| Control | 0 | 50.20 | 33.00 | 50.00 | 33.22 | 50.10 | 32.00 | 50.50 | 33.20 | 50.00 | 33.00 | 50.10 | 32.59 |

L.S.D. at 5%:

| | | | |
|---|---|---|---|
| Extracts (a) | : | 1.04 | 1.61 |
| Rates (b) | : | 1.94 | 1.22 |
| Isolates (c) | : | 2.25 | 2.44 |
| a x b x c | : | 3.33 | 4.14 |

*Continued.*

| Plant extracts | Rate g/l | *Candida* clinical isolates | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *Candida albicans* isolates | | | | | | *C. krusei* isolates | | | | | |
| | | (11) | | (12) | | (13) | | (11) | | (12) | | (13) | |
| | | Fresh weight (mg) | Dry weight (mg) | Fresh weight (mg) | Dry weight (mg) | Fresh weight (mg) | Dry weight (mg) | Fresh weight (mg) | Dry weight (mg) | Fresh weight (mg) | Dry weight (mg) | Fresh weight (mg) | Dry weight (mg) |
| *Saltera sarcocolla* | 20 | 42.00 | 31.43 | 41.88 | 32.44 | 42.35 | 32.39 | 44.00 | 33.10 | 44.10 | 32.89 | 43.99 | 33.23 |
| | 40 | 39.23 | 30.41 | 39.11 | 30.00 | 39.00 | 30.00 | 42.11 | 31.00 | 42.99 | 30.98 | 42.00 | 31.11 |
| $\overline{X}$ | | 40.61 | 30.92 | 40.49 | 31.32 | 40.63 | 31.19 | 43.05 | 32.05 | 43.54 | 31.93 | 42.99 | 32.17 |
| *Lepidium sativum* | 20 | 46.11 | 33.38 | 40.00 | 28.98 | 41.00 | 29.00 | 44.45 | 34.00 | 46.44 | 34.69 | 46.56 | 34.11 |
| | 40 | 43.40 | 32.50 | 36.70 | 24.00 | 37.00 | 23.89 | 46.45 | 32.00 | 45.00 | 32.23 | 45.11 | 32.17 |
| $\overline{X}$ | | 45.73 | 32.94 | 38.39 | 26.49 | 39.00 | 26.44 | 44.45 | 33.00 | 45.72 | 33.46 | 45.83 | 33.04 |
| Control | 0 | 51.25 | 34.20 | 51.10 | 35.89 | 50.76 | 35.58 | 50.11 | 35.20 | 50.00 | 35.10 | 50.12 | 35.00 |

L.S.D. at 5%:

| | | | |
|---|---|---|---|
| Extracts (a) | : | 1.92 | 2.78 |
| Rates (b) | : | 1.66 | 1.04 |
| Isolates (c) | : | 2.13 | 2.65 |
| a x b x c | : | 3.37 | 4.46 |

fungus and both tested extracts were noticed in their reaction. The efficiency of *S. sarcocolla* and *L. sativum* aqueous extracts as antifungal agents decreased with dilution. All *S. sarcocolla* and *L. sativum* plant extracts significantly reduced the mycelial growth (fresh weight only) of isolates of *C. parapsilosis* and *C. krusei*, but without significant differences between extracts or isolates of two fungi for dry weight as compared to control. On the other hand, extracts of the tested plants were more effective in reducing mycelial growth (dry and fresh weights) of *Candida albicans* isolates as compared to control. Also, increasing concentrations of the tested extracts caused significant reduction in growth of *C. albicans* isolates.

Generally, the efficiency of *S. sarcocolla* and *L. sativum* aqueous extracts as antifungal agents were more evident with high concentration. However, no significant differences between isolates of each fungus and extracts of the two plants species were observed. Data also showed that *S. sarcocolla* extract was more effective as antifungal agent against isolates of *Candida* species than *L. sativum* extract.

The antifungal activities of various natural substances have been reported by several investigators (Antonov *et al*., 1995; Arras *et al*., 1995 and Hassanein and El-Doksch, 1997). In the present study, higher activity of *S. sarcocolla* and *L. sativum* extracts against the fungal species, particularly at 40 g (all plant parts) of each of the two plant species. These results are in agreement with that reported by El-Safwani and Aly (2003). They found that the efficiency of thyme and peppemint aqueous extracts as antifungal decreased with dilution. In this concern, the effect of both extracts might be attributed to be antifungal activity of the natural components. Moreover, *S. sarcocolla* extract was more effective as antifungal agent against *Canaida* species than *L. sativum* extract might be due to its produced fungicidal effect and the wide spectrum of activity of this extract compared to that of *L. sativum*. Deepshikha *et al*. (2002) found that some by-products of this plant species (*L. sativum*) under investigation in this study such as benzylegaride and benzyliseothicynate which contribute towards its activity against *Bacillus subtilis* and micrococcus pyogenes. Zambonelli *et al*. (1996) found that the essential oil of thyme had strong antifungal activity against soil-borne fungi and this fungicidal activity was due to the active major constituent, thymol. Generally, it could be conclude that natural extracts of different medicinal plants exhibited antifungal activity against female genital tract inflammation disease. However, further experiments are needed in this field.

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## تأثير بعض المستخلصات النباتية على النمو الميسليومى لأنواع من فطر *Candida* معملياً

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تم دراسة تأثير المستخلص المائى لكل من نباتى الرشاد والعنزروت بتركيزين على النمو الميسليومى ( الوزن الجاف والطرى) لأنواع من فطر الكنديدا فى المعمل .
ولقد أظهرت النتائج أن التأثير المثبط لا يختلف فقط مع الأنواع الفطرية ولكن أيضاً مع المستخلصات النباتية :

- التركيزين 2% ،4% من المستخلصات النباتية كانا محل الدراسة لهما تأثير معنوى على كل الأنواع الفطرية المختبرة عند المقارنة بالكنترول .
- المستخلص المائى للعنزروت كان له تأثير أفضل من المستخلص المائى للرشاد على تثبيط النمو الميسليومى لأربع فطريات مستوردة وكذلك للعزلات المحلية لأنواع الكنديدا .
- المستخلصات النباتية لكلا النوعين النباتيين كانت أكثر تأثيراً على النمو الميسليومى للفطر *C. albicans* مقارنة بعزلات الكنديدا الأخرى والكنترول .
- كلما زاد التركيز المستخدم من المستخلص النباتى المختبر أدى إلى تقليل النمو الميسليومى للفطريات محل الدراسة بدرجة معنوية .