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*Full Length Research Paper*

# Anticandidal screening and antibacterial of *Citrullus colocynthis* in South East of Iran

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**Resistance to current antibacterial drugs and the rise of opportunistic fungal infections are growing global concerns. Traditional medicine is a potential source of new antibacterials and antifungals. *Citrullus colocynthis* Schrad (Cucurbitaceae) endemic in Southern Iran is used in folk medicine against dermatological, gynaecological and pulmonary infections. To assess *in vitro* antibacterial and anticandidal activity of aqueous and diluted acetone extracts of *C. colocynthis* Schrad. MIC and MBC/MFC were determined for plant organs at different maturation stages. *C. colocynthis* Schrad. was harvested and its identification was verified. Aqueous and diluted acetone extracts (from the plant's roots, stems, leaves and three maturation stages of its fruit and seeds) were screened for activity against Gram-negative and Gram-positive bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Enterococcus faecalis*) and various *Candida* spp. (*Candida glabrata*, *Candida albicans*, *Candida parapsilosis* and *Candida kreusei*). All extracts showed activity against all strains. The highest MICs and MBCs/MFCs were obtained from the fruit aqueous extracts (MIC 0.10 mg/ml against *C. albicans* and *C. glabrata*, 0.20 mg/ml against *E. coli* and *P. aeruginosa*), lowest activity from the root extracts. *C. colocynthis* Schrad shows antibacterial and anticandidal properties. The folk medicinal use as a broad-spectrum antimicrobial agent is validated.**

**Key words:** *Citrullus colocynthis* Schrad, aqueous extract, acetone extract, antibacterial, anticandidal, Iran.

## INTRODUCTION

The use of plants for medicinal purposes has been practiced for many centuries by a substantial proportion of Iran's population. Interest in ethnopharmacy as a source of pharmacologically active compounds has increased worldwide, particularly in the search for drugs to counter multi-resistant microorganisms. Additionally, in some developing countries, plants are the main medicinal source to treat infectious diseases due to economic conditions and availability. However, only approximately 20% of the plants found in the world have been submitted to pharmacological or biological testing, despite the substantial number of new antibiotics derived from natural or semi-synthetic resources being introduced on the market (Mothana and Lindequist, 2005). *Citrullus colocynthis* Schrad. (Cucurbitaceae), growing in arid areas, is endemic in the South of Tunisia (Pottier-Alapetite, 1981). In Iran, as in the rest of the persian gulf (Mozaffarian, 1964), the parts of plants most often used for medicinal purposes are fruits and/or seeds, though other parts of the plants can be used, for example roots to treat urinary infection (Nadkami, 1954) or leaves (Batanouny, 1999). Traditional healers seem to not pay attention to the plant's degree of maturity. The literature rarely mentions if seeds are present in preparations involving ground fruit/pulp. Modes of preparation and administration vary, even for similar indications. Common preparations use fresh, warmed or dried plant material (often ground), as well as extracts used mostly in a liquid form. Extracts are prepared either in water or in aqueous mixtures containing more lipophilic compounds (hot milk extractions, water/olive oil at various ratios) at a range from room temperature (maceration) to boiling. The use of *C. colocynthis* Shrad oil (expressed from the fruit or

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**Table 1.** Extraction yields (w/w%) and phytochemical screening of *Citrullus colocynthis Schrad.*

| Variable | Extraction yields | | Phytochemical screening of Citrullus colocynthis Schrad. | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Alkaloids | | Coumarins | | Flavonoids | | Anthraq. | | Cardiac glyc | | Iridoids | | Saponids | | Steroids | | Gallic tanins | |
| | Aq. | Ac. | Aq. | Ac. | Aq. | Ac. | Aq. | Ac. | Aq. | Ac. | Aq. | Ac. | Aq. | Ac. | Aq. | Ac. | Aq. | Ac. | Aq. | Ac. |
| Roots | 4.558 | 2.630 | – | – | – | – | – | – | – | – | – | – | + | – | – | – | + | + | – | – |
| Leaves | 12.887 | 7.014 | + | + | + | + | – | – | – | – | – | – | + | – | – | – | + | + | + | + |
| Stems | 11.047 | 8.266 | + | + | – | – | – | – | + | – | – | – | + | – | – | – | + | + | – | – |
| Immature fruit | 2.758 | 2.611 | + | + | – | – | – | – | – | – | – | – | + | – | – | – | + | + | – | – |
| Ripening fruit | 2.907 | 2.980 | + | + | – | – | – | – | – | – | – | – | + | – | – | – | + | + | – | – |
| Ripe fruit | 3.041 | 3.075 | + | + | – | – | – | – | – | – | – | – | + | – | – | – | + | + | – | – |
| Immature seed | 2.937 | 3.032 | + | + | – | – | + | + | – | – | – | – | – | – | – | – | + | + | – | – |
| Ripening seed | 2.750 | 2.850 | + | + | – | – | + | + | – | – | – | – | – | – | – | – | + | + | – | – |
| Ripe seed | 2.212 | 2.275 | + | + | – | – | + | + | – | – | – | – | – | – | – | – | + | + | – | – |

Aq.: aqueous extract; Ac.: diluted acetone extract; Antraq.: anthraquionones; Cardiac glyc.: cardiac glycosides; +: presence; –: absence.

distilled) is not documented in Iran. This is all the more notable that family-sized oil presses and stills (Clevenger-type apparatus) for essential oils are widespread.

Ground plant material can be mixed with honey for ingestion or topical gynaecological application or with other plants for poultices (for example with *Lawsonia inermis* and *Capparis spinosa*). Methods of administration are topical, rectal or vaginal suppositories (fruit), enema, cervico-vaginal douche, and by ingestion (Boukef, 1986; Marzouk, 2008). Extreme caution should be exercised with ingestion, due to the plant's drastic laxative properties, and with contact with leaves, due to risks of syncope (for all mammals including domestic animals) (Marzouk, 2008). Use is contraindicated during pregnancy as the plant is abortifacient (Pottier-Alapetite, 1981; Delazar et al., 2006). Many of today's traditional medicinal uses of the plant are found throughout history (Eber's papyrus in ca.1550 BCE Egypt) (Riddle, 1999) over a large geographical zone from Mauritania to India, even extending outside of the plant endemic zone, to Europe (Adams et al., 2009). The current study measured in vitro antibacterial and anticandidal activities of this plant using the broth serial dilution (microdilution method). Plants were collected and identified. Then, preparation and testing were carried out on the reconstituted lyophilized extracts of aqueous and diluted acetone extracts from the roots, stems and leaves, as well as three different maturation stages of the fruit and seeds.

## MATERIALS AND METHODS

### Plant material

*C. colocynthis* Schrad plants were collected in south east of iran in zabol city. The identification was performed according to the flora of Iranica (Ghahraman, 1981) and a voucher specimen (C.C-01.01) deposited in the biological laboratory of the Faculty of Pharmacy of Monastir.

### Extraction protocol

The extraction was performed on 100 g of fresh organs: Roots, stems, leaves and three different stages of maturation of fruits and seeds (immature, ripening and ripe). Yields of prepared extracts are given in Table 1.

#### *Aqueous extract*

One hundred grams of fresh organs were ground with a mixer and added to 500 ml of distilled water. The mixture was allowed to reflux for 30 min, after which the solution was allowed to cool (4 h at 3°C). The mixture was then filtered using filter paper (Whatman no.1) under the vacuum of a water pump. The filtrate obtained was lyophilized, yielding the lyophilized aqueous extract.

#### *Diluted acetone extract*

To 100 g of fresh organs were added 500 ml of an acetone-water mixture (3/4 vol. acetone completed with distilled water). The mixture was ground with a mixer, followed by reflux for 30 min, after which the solution was allowed to cool (24 h at 3°C). A precipitate formed, which was removed by filtering (Whatman paper no.1). The filtrate was concentrated by rotavapory extraction to remove the acetone. A new precipitate appeared in the concentrated liquid and was filtered out. The final filtrate was lyophilized, giving the lyophilized diluted acetone extract.

**Table 2.** Antibacterial MIC (mg/ml) and MBC (mg/ml) of lyophilized aqueous extracts.

| Variable | *E. coli* ATCC 25922 | | *P. aeruginosa* ATCC 27853 | | *S. aureus* ATCC 25923 | | *E. faecalis* ATCC 29212 | |
|---|---|---|---|---|---|---|---|---|
| | MIC | MBC | MIC | MBC | MIC | MBC | MIC | MBC |
| Roots | 3.25 | 6.50 | 3.25 | 6.50 | 3.25 | 6.50 | 3.25 | 6.50 |
| Leaves | 1.63 | 3.25 | 0.81 | 1.63 | 1.63 | 3.25 | 1.63 | 3.25 |
| Stem | 1.63 | 3.25 | 0.81 | 1.63 | 0.81 | 1.63 | 1.63 | 3.25 |
| Immature fruit | 0.20 | 0.41 | 0.23 | 0.41 | 0.41 | 0.81 | 0.81 | 1.63 |
| Ripening fruit | 0.41 | 0.41 | 0.81 | 1.63 | 3.25 | 3.25 | 0.81 | 3.25 |
| Ripe fruit | 0.20 | 0.41 | 1.63 | 3.25 | 3.25 | 3.25 | 1.63 | 3.25 |
| Immature seed | 0.41 | 0.81 | 0.81 | 1.63 | 0.81 | 1.63 | 0.81 | 1.63 |
| Ripening seed | 0.81 | 1.63 | 1.63 | 3.25 | 1.63 | 3.25 | 1.63 | 3.25 |
| Ripe seed | 0.81 | 1.63 | 1.63 | 3.25 | 1.63 | 3.25 | 3.25 | 6.50 |

MIC positive control: Levofloxacin (*E. coli* 0.61 µg/ml, *P. aeruginosa* 0.3 µg/ml, *S. aureus* 0.3 µg/ml, *E. faecalis* 1.22 µg/ml). *E. coli: Echerichia coli; P. aeruginosa: Pseudomonas aeruginosa; S. aureus: Staphylococcus aureus; E. faecalis: Enterococcus faecalis*.

**Table 3.** Antibacterial MIC (mg/ml) and MBC (mg/ml) of lyophilized acetone extracts.

| Variable | *E. coli* ATCC25922 | | *P. aeruginosa* ATCC 27853 | | *S. aureus* ATCC2593 | | *E. faecalis* ATCC29212 | |
|---|---|---|---|---|---|---|---|---|
| | MIC | MBC | MIC | MBC | MIC | MBC | MIC | MBC |
| Roots | 6.50 | ND | 6.50 | ND | 6.50 | 6.50 | 6.50 | 6.50 |
| Leaves | 3.25 | 6.50 | 0.41 | 0.81 | 0.41 | 0.81 | 0.41 | 0.81 |
| Stem | 3.25 | 6.50 | 0.41 | 0.81 | 1.63 | 3.25 | 0.81 | 1.63 |
| Immature fruit | 0.41 | 0.81 | 0.41 | 0.40 | 0.81 | 1.63 | 0.81 | 1.63 |
| Ripening fruit | 0.41 | 0.81 | 0.81 | 1.63 | 0.81 | 1.63 | 0.81 | 1.63 |
| Ripe fruit | 1.63 | 3.25 | 1.63 | 3.25 | 1.63 | 3.25 | 1.63 | 3.25 |
| Immature seed | 0.81 | 1.63 | 0.81 | 1.63 | 1.63 | 3.25 | 1.63 | 3.25 |
| Ripening seed | 1.63 | 3.25 | 0.81 | 1.63 | 1.63 | 3.25 | 1.63 | 3.25 |
| Ripe seed | 1.63 | 3.25 | 1.63 | 3.25 | 3.25 | 6.50 | 3.25 | 6.50 |

MIC positive control: Levofloxacin (*E. coli* 0.61 µg/ml, *P. aeruginosa* 0.3 µg/ml, *S. aureus* 0.3 µg/ml, *E. faecalis* 1.22 µg/ml). *E. coli*: *Echerichia coli; P. aeruginosa: Pseudomonas aeruginosa; S. aureus: Staphylococcus aureus; E. faecalis: Enterococcus faecalis*. ND: not determined.

**Qualitative phytochemical screening**

Each extract was screened for the presence of key families of phytochemicals (Sakar and Tanker, 1991; Trim and Hill, 1952) using the following reagents and chemicals: alkaloids with Dragendorff's reagent confirmed with Bouchardat's ($I_2$/$MgI_2$) and with Meyer's reagents (KI/$MgCl_2$), coumarins with diluted NaOH-U.V. test, flavonoids with metallic magnesium and hydrochloric acid (HCl), anthraquinones with Borntrager's reagent, cardiac glycosides with Kedde's reagent (and confirmed with Baljet's reagent), iridoids with diluted hydrochloric acid, saponids for their ability to produce suds, steroids with acetic anhydride and concentrated sulphuric acid (Liebermann reaction), tannins in general with ferric chloride (confirmed with concentrated hydrochloric acid, Bath-Smith reaction) and gallic tannins specifically with Stiasny reagent (Table 1).

**Antibacterial and anticandidal activity**

***Microorganisms***

Four reference strains were chosen for antibacterial investigation: Gram-positive cocci (*Enterococcus faecalis* ATCC 29212 and *Staphylococcus aureus* ATCC 25923) and Gram-negative bacilli (*E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853). The antifungal effect of the various *C. colocynthis* Schrad. extracts was also tested against a range of pathogenic reference yeasts (*Candida albicans* ATCC 90028, *Candida glabrata* ATCC 90030, *Candida kreusei* ATCC 6258 and *Candida parapsilosis* ATCC 22019).

**MIC and MBC/MFC determinations**

The minimal inhibitory concentration (MIC) preventing visible bacterial or fungal growth was measured by the broth dilution method (microdilution using 96-well microplates), following the procedure of Berche et al. (1991). All extracts stock solution were prepared by dissolution in 10% dimetyl sulfoxyde (DMSO). The plant extracts concentrations tested ranged from 0.10 to 6.50 mg/ml. The MIC of each extract was defined as the lowest concentration which inhibited either bacterial or candidal growth, after incubation at 37°C between 18 and 24 h. The minimal bactericidal concentration (MBC) and the minimal fungicidal concentration (MFC) were determined by subculture on blood agar at 37°C between 18 and 24 h. Levofloxacin was used as

**Table 4.** Antifngal MIC (mg/ml) and MFC (mg/ml) of lyophilized aqueous extracts.

| Variable | *C. albicans* ATCC 90028 | | *C. glabrata* ATCC 90030 | | *C. kreusei* ATCC 6258 | | *C. parapsilosis* ATCC 22019 | |
|---|---|---|---|---|---|---|---|---|
| | MIC | MFC | MIC | MFC | MIC | MFC | MIC | MFC |
| Roots | 3.25 | 6.50 | 3.25 | 6.50 | 3.25 | 6.50 | 3.25 | 6.50 |
| Leaves | 1.63 | 3.25 | 0.81 | 1.63 | 1.63 | 3.25 | 0.81 | 1.63 |
| Stem | 1.63 | 3.25 | 1.63 | 3.25 | 1.63 | 3.25 | 0.81 | 1.63 |
| Immature fruit | 0.41 | 0.81 | 0.41 | 0.81 | 0.41 | 0.41 | 0.41 | 0.81 |
| Ripening fruit | 0.41 | 0.41 | 0.20 | 0.20 | 0.20 | 0.41 | 0.41 | 0.41 |
| Ripe fruit | 0.20 | 0.41 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.41 |
| Immature seed | 0.20 | 0.41 | 0.20 | 0.81 | 0.81 | 1.63 | 0.20 | 0.41 |
| Ripening seed | 0.81 | 1.63 | 1.63 | 3.25 | 0.81 | 1.63 | 0.81 | 1.63 |
| Ripe seed | 0.81 | 1.63 | 3.25 | 6.50 | 3.25 | 6.50 | 0.81 | 1.63 |

Positive control with Amphotericin B (MFC 0.5 µg/ml). *C. albicans: Candida albicans; C. glabrata: Candida glabrata; C. kreusei: Candida kreusei; C. parapsilosis: Candida parapsilosis.*

**Table 5.** Antifungal MIC (mg/ml) and MFC (mg/ml) of lyophilized acetone extracts.

| Variable | *C. albicans* ATCC 90028 | | *C. glabrata* ATCC 90030 | | *C. kreusei* ATCC 6258 | | *C. parapsilosis ATCC 22019* | |
|---|---|---|---|---|---|---|---|---|
| | MIC | MFC | MIC | MFC | MIC | MFC | MIC | MFC |
| Roots | 6.50 | ND | 3.25 | 6.50 | 6.50 | ND | 3.25 | 6.50 |
| Leaves | 0.81 | 1.63 | 0.81 | 1.63 | 0.41 | 0.81 | 0.81 | 1.63 |
| Stem | 1.63 | 3.25 | 0.20 | 0.41 | 3.25 | 6.50 | 0.81 | 1.63 |
| Immature fruit | 0.10 | 0.20 | 0.10 | 0.20 | 0.20 | 0.41 | 0.20 | 0.41 |
| Ripening fruit | 0.41 | 0.81 | 0.41 | 0.81 | 0.20 | 0.41 | 0.20 | 0.41 |
| Ripe fruit | 1.63 | 3.25 | 0.41 | 0.81 | 0.81 | 1.63 | 1.63 | 3.25 |
| Immature seed | 0.41 | 0.81 | 0.81 | 1.63 | 0.41 | 0.81 | 0.41 | 0.81 |
| Ripening seed | 0.81 | 1.63 | 0.81 | 1.63 | 0.81 | 1.63 | 0.41 | 0.81 |
| Ripe seed | 1.63 | 1.63 | 1.63 | 3.25 | 1.63 | 3.25 | 0.81 | 1.63 |

Positive control with Amphotericin B (MFC 0.5 µg/ml). *C. albicans: Candida albicans; C. glabrata: Candida glabrata; C. kreusei: Candida kreusei; C. parapsilosis: Candida parapsilosis.* ND: not determined.

antibacterial positive control, and Amphotericin B for the anticandidal one. Results are presented in Tables 2, 3, 4 and 5 with the highest activities for each type of extraction in various shades of grey and the two most noticeable antibacterial and anticandidal activities against different strains are shown in bold face.

## RESULTS

### Extraction yields

Leaves and stems have significantly much higher extraction yields than other plants parts (both for aqueous and acetone extraction). Aqueous extraction gave higher yields than the acetone extraction for roots, leaves and stems (Table 1). The extraction yields were close for both methods with immature fruits, but acetone extraction gave better yields for ripening and ripe fruits and seeds in all their stages.

### Qualitative phytochemical screening

Results (reported in Table 1) were dependent upon the extraction method: aqueous extracts contained iridoids and anthraquinones not detected in diluted acetonic ones. Differences were also noted between plant organs: Alkaloids were found in all extracts except the roots, flavonoids were present only in seeds, gallic tannins and coumarins only in leaves, anthraquinones only in (aqueous) stems extracts and iridoids in all aqueous extracts but the ones from seeds. However, no extract contained saponids and all of them contained steroids.

**Antibacterial activity**

All the extracts tested from Tunisian *C. colocynthis* Schrad showed antibacterial activity against all tested strains. MIC and MBC were tested for concentrations ranging from 0.10 to 6.50 mg/ml (Tables 2 and 3) and all plant organs exhibited antibacterial activity against all tested strains. Gram-negative bacilli were more sensitive than the Gram-positive. The plant organs with the highest antibacterial properties were immature fruits and immature seeds (in both cases their aqueous as well as their diluted acetone extracts). The most active organs of all were immature fruits. The strongest inhibitions were obtained against *E. coli* and *P. aeruginosa* with aqueous extracts of immature fruits. For both strains, the MIC was of 0.20 mg/ml and the MBC of 0.40 mg/ml. Stem extracts were as active as those of leaves, these two organs showing generally less activity than the fruits and seeds. The lowest activity (against all strains) was observed for root extracts (MIC ranging from 3.25 to 6.50 mg/ml).

**Anticandidal activity**

Anticandidal activity is reported as MIC and MFC (Tables 4 and 5). With the exception of roots, all extracts showed significant antifungal activity against all tested yeasts. Overall, the best antifungal activity was against *C. albicans* and *C. glabrata* (for both aqueous and diluted acetone extracts). In terms of plant organs, the best activities were found for fruits at one or more of their ripening stages (either immature for the acetone extracts, or ripening and/or mature for the aqueous extracts).

For the aqueous extracts, mature fruits had the highest anticandidal effects in all strains (MIC 0.20 mg/ml). Immature seeds were as efficient as mature fruits for *C. albicans*, *C. glabrata* and *C. parapsilosis*. Ripening fruits (aqueous extracts) were either as strongly anticandidal as mature fruits, for *C. glabrata* and *C. krensei*, or a close second (MIC 0.40 mg/ml).

For acetone extracts, immature fruits were the most active against all strains (with the best activity against *C. albicans* and *C. glabrata*, MIC 0.10 mg/ml). Ripening fruits were either as efficient as immature ones (*C. krensei*, *C. parapsilosis*, both MIC and MFC) or had the second best efficacy against *C. albicans*. For *C. glabrata*, the stems showed the second best and very active anticandidal property. The activity of the stem diluted acetone extract against *C. glabrata* differs significantly from its activity against other strains, for which its activity ranks below that of other organs (seeds, leaves). Note also that this result is specific to the acetone extracts, as aqueous extracts from leaves showed better activity than the one from stems.

**DISCUSSION**

Tunisia recently increased research in Traditional Herbal Medicines following scientific confirmation of their effectiveness in treating conditions for which they were traditionally prescribed. The present investigation has explored the use of one such plant, *C. colocynthis* Schrad., endemic in the south of Tunisia, for treating infectious diseases.

The antimicrobial and anticandidal screening showed interesting activities at low concentrations: Every plant organ was active, except roots. Plant extracts are generally a crude mixture of non-active and active compounds and their MICs must be interpreted accordingly. For example, in a discussion on anticandidal properties of Canadian medicinal plant extracts (Carmeli et al., 1999), the MIC of *Epilobium augustifolium* at 0.80 mg/ml against *C. albicans* ATCC 90028 was interpreted as strong antifungal activity: The authors noted that MICs of less than 1.00 mg/ml should be interpreted as strong antifungal potential (comparable to accepted active antimicrobial single products such as cationic peptides in the 0.10 mg/ml range). MICs obtained in this study (as low as 0.10 mg/ml) are within the range of what is considered significant for plants and even purified extracts.

The study clarified a complex ethnopharmacological picture in terms of preparation: Firstly, results showed the importance of maturation: At different stages of maturation, the compositions of fruits and seeds differed, as made obvious by their extraction yields and antimicrobial and anticandidal activities. Which maturation stage was the most efficient depended on the extraction method (for example compare results of active fruits in Tables 4 and 5).For a given microorganism, the method of extraction can also change which plant organ is the most effective (for *S. aureus* and *E. faecalis*, the best antimicrobial plant organ extracts changes from immature fruits in aqueous extracts to the stronger activity in of leaves in the diluted acetone extracts). In addition, the difference in activity between the de-seeded fruits and the seeds emphasizes the need for precise recording of ethnobotanical protocols to notice if seeds are present in preparations involving “pulp” or “fruit”.

For the antimicrobial activity, the plant extracts were active against both Gram-positive and Gram-negative bacteria, though more against the latest. Activity depended from the bacterial strain, the plant organ, their maturation state and the nature of the extraction.

The good MIC values (0.20 mg/ml) against *E. coli* of an extract obtained from a productive organ through an easy extraction procedure (fruit, aqueous extraction, good extraction yields) bodes well for widespread potential applications. The need to decontaminate surfaces soiled with *E. coli* is of key economic importance in the health care and industrial sectors (for example the meat packing industry). Novel anti *P. aeruginosa* activity is also of particular interest as it is the leading cause of nosocomial infections and has developed mechanisms of resistance to common antibiotic classes (Carmeli et al., 1999). In the same way, synergy between commercial drugs showed

promising results for a complicated urinary infection with *P. aeruginosa* (Hayami et al., 1999) and FIBs between plants products or their various organs are getting more thoroughly investigated (Van Vuuren and Viljoen, 2008), active extracts of *C. colocynthis* Schrad. should be tested for potential synergy with other antimicrobials.

Activity cannot be imputed to one family of phytochemicals only (or its absence). Alkaloids are commonly found to have antimicrobial properties (Omulokoli et al., 1997); therefore their absence in roots could account for the lack of activity of this organ. However, alkaloids cannot be solely responsible for the activity: For the same organ, the majority of aqueous extracts are more active than the acetone ones, which could reflect preferential solubility/extraction properties of the alkaloid(s) in water versus acetone, but the diluted acetone extracts of leaves are more active than their aqueous extracts for 3 out of 4 strains. Therefore, activity is not due to the alkaloids alone. Flavonoids (detected in all the seeds extracts) are known to be synthesized by plants in response to microbial infection (Fogliani et al., 2005); therefore their potential in vitro antimicrobial effectiveness against a wide array of microorganisms should not come as a surprise, but activity of immature seeds (not containing them) and the low or near lack of activity of ripe fruit (which contain them) point to other factors at play. The same could be said about most phytochemicals detected by the qualitative chemical analysis and known from works in other plant species to have antimicrobial pharmacological activity: tannins (Ayaz et al., 2008), steroids (Khan et al., 2007), pigments (Fogliani et al., 2005; Eyong et al., 2006) and flavonoids (Fogliani et al., 2005), alkaloids (Yan et al., 2008) and iridoids (Akunvili et al., 1991). Iridoids, present in the aqueous samples only, could contribute to their better performance compared to the acetone extracts. However, iridoids are detected in the aqueous root extract but this extract showed no overall activity. Therefore, iridoids role, if any, is likely to be synergistic or potentiate other compounds.

Concerning the anticandidal activity, the present study showed MICs and MFCs significant for crude plant extracts against all strains. The potent activity against *C. albicans* (MIC 0.10 mg/ml for acetone extracts – bold face Table 5 – and 0.20 mg/ml for some aqueous extracts, Table 4) is particularly welcomed due to the fungus' frequency to cause infections, with ca. 45% of clinical fungal infections due to this strain (Gupta et al., 2004). The equally strong activity against *C. glabrata* opens the possibility to use the extracts either alone or in combination with Amphotriricin B, as has been proposed with other natural substance extracts (Mimee et al., 2005). New active drugs are particularly needed as triazoles are generally met with resistance and the few alternative treatments, such as Caspofungin, are limited by their low oral bioavailability (Boucher et al., 2004). Like for the antimicrobial activity, the anticandidal activity may be attributed, possibly in combination, to various phytochemicals detected during the extracts chemical screening and which are known to cause damage to cell membranes, causing leakage of cellular materials and ultimately the microorganisms death (Mshvildadze et al., 2000; Ghani et al., 2008; Abdel et al., 2008). The trend over all the strains to have higher activity for ripe fruits aqueous extracts versus immature fruit for the diluted acetone extract points towards various active, potentiate or antagonistic compounds present in various concentrations according to the maturation stage of the fruit and which can be preferentially extracted with various solvents. It invites and in-depth study of the most active organs contents to determine (by MICs and FICs) if further purified extracts or isolated substances, alone or in combination, could display an even higher activity and/or selectivity against specific microorganisms.

With all these wide spectrum antibacterial and anticandidal properties, *C. colocynthis* Schrad can be considered an effective antimicrobial agent to treat infectious diseases. This plant, namely its fruit extracts, demonstrated activity against some bacteria and fungi prevalent in dermatology, gynaecological and pulmonary infections. The study supported scientifically the ethnopharmacological use of the plant as an antimicrobial and anticandidal agent and could account for some of the variations observed in the ethnopharmaceutical preparation methods. Therefore, the use of this plant as antimicrobial agent is validated by the results obtained in this work. Further studies are ongoing to identify the chemical compounds of these antimicrobial extracts.

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