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Evaluating the efficacy of flavonoids from *Capparis spinosa* extracts in treatment of *Candida vulvovaginitis*

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ABSTRACT

Flavonoids were extracted from the leaves and stems of the *Capparis spinosa* (Caper) plant. The total flavonoids were detected with qualitative and quantitative assays. *C. albicans* isolates were isolated from patients infected with vaginal candidiasis and these fungal isolates revealed high levels of biofilm formation. Flavonoids isolated from the leaves and stems of the *C. spinosa* plant were found to have antibiofilm activity against all *C. albicans* isolates dependent on dose manner. In comparison to the control, flavonoids influence all *C. albicans*, at 400 µg mL^{-1}, with percentages of biofilm inhibition ranging from 69 to 31%. These findings imply that flavonoids could be useful in treating biofilm-associated *C. albicans* infections to overcome the difficulties associated with multi-antibiotic resistance.

Keywords: *Capparis spinose, Candida vulvovaginitis*, Flavonoids.
Article type: Research Article.

INTRODUCTION

Capparis spinosa (Caper) can be found growing in the arid Gobi Desert or on stony mountains. *C. spinosa* is divided into two subspecies: subsp. *spinosa* and subsp. *Rupestris* (Fici 2014). Since ancient times, *C. spinosa* has been utilized as a traditional herbal cure for its positive benefits on human ailments, including its fruits, roots, and other parts. In traditional Chinese medicine, *C. spinosa* has traditionally been used to treat rheumatoid arthritis and gout (Ao *et al.* 2007). In Pakistan's northern regions, the root barks of *C. spinosa* have been used to treat splenomegaly, psychiatric diseases, and tubercular glands (Afzal *et al.* 2009). *C. spinosa* extracts, according to (Zou *et al.* 2006), are a very potent source of antifungal and anti-inflammatory properties *C. spinosa* was utilized to prepare herbal tea. Buds and leaves of *C. Spinosa* are commonly used to cure colds and other ailments, as well as gastrointestinal infections, diarrhea, and dysentery, and to remove kidney stones (Sher & Alyemeni 2010). *C. spinosa* roots were used to cure liver and renal problems in Egypt and the Arab area, as well as paralysis and diabetes in the Ancient Romans and Moroccans (Tlili *et al.* 2011). *C. spinosa* extracts were studied for their antihypertensive, poultice, anti-hepatotoxic, and anti-allergic properties also used to treat hemorrhoids and gout (Mahboubi & Mahboubi 2014). Due to an increase in the number of patients receiving immunosuppressive

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therapy, increased major surgeries and broad-spectrum antibiotherapy, hyperalimentation, and prolonged intensive care unit stays for patients with poor health status, the prevalence of serious fungal infections, particularly invasive *Candida* infections, has increased in recent years (Lass Flörl 2009). More than half of all human candidiasis is caused by *C. albicans*, which causes two types of infections: superficial infections (nonlethal), such as oral or vaginal candidiasis, and systemic infections (Leon *et al.* 2014). *Candida* spp. is the most common cause of vulvovaginitis in women, with *C. albicans* being the most common *Candida* spp. *C. albicans* contains a particular collection of proteins (adhesins) that mediate adhesion to other *C. albicans* cells, other microbes, abiotic surfaces, and host cells (Garcia *et al.* 2011). The agglutinin-like sequence (ALS) proteins, which compose a family of eight members (ALS1–7 and ALS9), are responsible for *C. albicans* adhesions. Hwp1, a hypha-associated protein, is another essential *C. albicans* adhesion (Zordan & Cormack 2012). As a result, there was a need to look into the utilization of flavonoids extracted from the plant *C. spinose* in the treatment of *Candida* spp. which is the most common cause of vulvovaginitis in women.

MATERIALS AND METHODS

Plant Collection

Fresh *Capparis spinosa* plants (leaves and steams) were taken from some Dhiala agricultural sites. The aerial piece was finely pulverized using an electric grinder after being dried at room temperature in the dark for 10 days.

Preparation of crude extracts

Two hundred gram dried aerial component powder (leaves and steams) was macerated in 1-L 70% ethanol for 72 h in a dark environment, then filtered and concentrated with a rotary evaporator (Harborne 1984)

Detection of Flavonoids

Alkaline reagent test: Sodium hydroxide solution is combined with a few drops of plant extract solution and left for a few minutes.

Total flavonoids extraction from the plant *C. spinosa*

About 200 g of fresh plant samples were placed in a 1-L glass flask, 600 mL of distilled water with (10% v/v) HCl was added, and then reflex extraction was performed for 8 hours continuously to ensure that the glycoside linkage between the flavonoids and aglycon parts was cleaved and broken. Filtered and cooled plant extracts were used. Using an organic solvent such as ethyl acetate, the aglycon parts of flavonoids, which constitute the biologic active part, were extracted by adding 50 mL for each 50 mL extract and repeating three times with a separating funnel. The plant's acetate layers were gathered in the separating funnel once more, and an equivalent amount of distilled water was added to eliminate the HCl residues left over from the extraction. A rotary evaporator was used to dry the acetate layer at 45 °C. To finish the rest of the analysis, the plant's output was weighted and saved (Harborne 1984).

Quantitative assay for total flavonoids

In a 50% ethanol solution, several quercetin standard flavonoids solutions were produced with concentrations of 0.2, 0.4, 0.6, 0.8, and 1 mg mL^{-1}. The following interaction was carried out. An aliquot of 1 mL of stock total flavonoids extract solution (5 mg mL^{-1}) was transferred to a glass tube, and 1 mL Querecetin standard solutions of each concentration were placed in a separate glass tube, then 1 mL of 5% sodium nitrite solution was added to all tubes, stirred, and left at room temperature for 5 minutes. Then 1.5 mL aluminum chloride 10% was added to all tubes and mixed well, and finally, 5 mL. To derive the straight-line equation, a standard curve was drawn between the absorbance readings of each standard solution versus their concentration. The concentration of total flavonoids in the extract was determined as follows: $Y = 0.4618 x$ (Wang *et al.* 2009).

Samples collection and isolation

High vaginal swabs were taken from 50 patients who had vulvovaginal candidiasis and were between the ages of 18 and 50. These swabs were separated into two smears: one was viewed under a microscope right away for direct examination, while the other was normally cultured on an SDA medium.

Identification of Candidal Isolates

Purified isolates were streaked on Sabouraud dextrose agar using ABC techniques, then incubated at 37 °C for two days to obtain one isolated pure colony. *Candida* was recognized based on morphological features on culture

medium and germ tube formation providing the diagnosis by detecting *Candida* spp. This isolated colony was transferred to SDA by streaking the entire plate and then incubated at 37 ºC overnight. Using the Vitek 2 system.

Biofilm formation assay

Biofilm formation was measured in this investigation using pre-sterilized polystyrene 96-well microplates using a modified approach reported by Melek *et al*. (2012). Yeast was inoculated using a loop into a tube containing 2 mL of YPD broth and incubated at 37°C for 24 hours. Thereafter, all tubes were diluted at a ratio of 1:20 with freshly made YPD with 1% glucose, and each well of the microplate was filled with 200 L of the final solution. Microplates were sealed with covers and incubated for 24 hours at 37 °C. The media in the wells was removed and washed twice with sterile phosphate buffer solution (PBS), then it was inverted to blot and dried. Microplates were stained by adding 200 L of 0.1g 100 mL^{-1} crystal violate to each well, incubating for 20 min, then washing twice with PBS, inverting to blot, and allowing to dry. Finally, 200 L of acetone: ethanol mixture (20:80 v/v) was added to each well, waiting for about 10 minutes, then reading at 450 nm with an Elisa reader.

Biofilm inhibition assays

The extracted flavonoids were put to the test to see if they might prevent biofilm development in the biofilm-producing *Candida* isolates. At the moment of inoculation, flavonoids concentrations were added to the growing medium, and the cells were allowed to develop a biofilm. Fungal suspensions were combined with various flavonoids concentrations ranging from 50 to 400 µg mL^{-1}, as well as water as a control, before being transferred to the plate. After incubation at 37 °C for 24 hours and completion of the experiment as described above, the percentage of biofilm inhibition was determined using Marak & Dhanashree (2018): Percentage of biofilm inhibition (%) = [OD control-OD treatment]/OD control × 100

RESULTS AND DISCUSSION

Preparation of ethanolic crude extract and quantitative screening of flavonoids formation

About 200 g of plant components yielded 17.43 grams of crude residue, which was labeled as such. The alkaline reagent test for flavonoids yielded (++) with the production of bright yellow color.

Qualitative screening of flavonoids formation

Based on the standard curve for different concentrations of the standard quercetin (Fig. 1), the total quantity of flavonoids found in the plant's leaves and stems was assessed, and it was discovered that 200 g dried aerial section of the plant had 426.87 mg mL^{-1} total flavonoid.

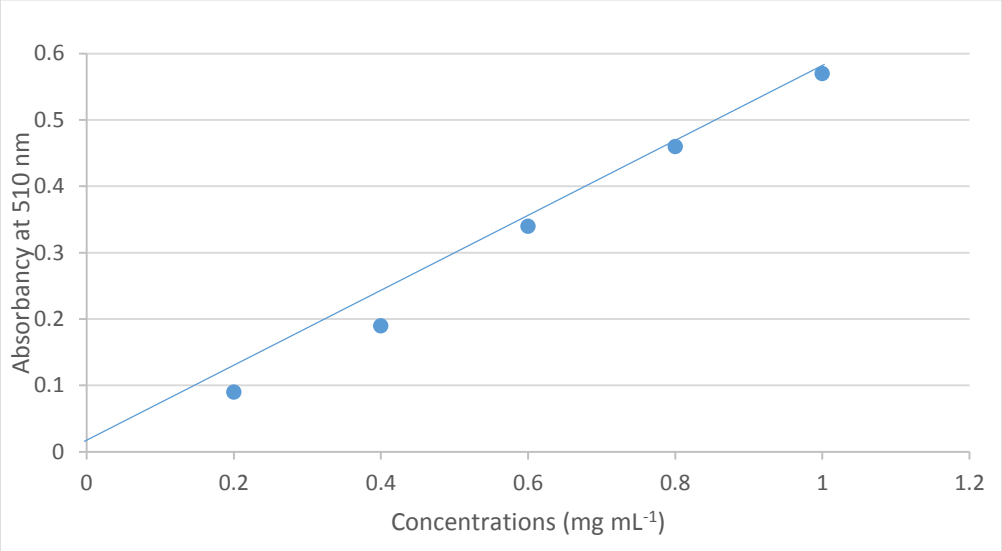

Fig. 1. Standard curve for different concentrations of the standard quercetin.

Polyphenols and flavonoids were abundant in *C. spinosa* leaves and flowers, but not in the roots (Qayoom Mir *et al.* 2009; Zhou *et al.* 2011; Salhi *et al.* 2019). The amount of quercetin in various plant sections was measured at the ripe fruiting stage of *C. spinosa*. The quantity of quercetin in various parts of the caper ranged from 1.7 mg g^{-}

^{1} to 12.8 mg g^{-1}, with the amount of quercetin in the flower, floral bud, and fruit being higher than all other parts. The highest rutin level was found in the leaves. According to Yang *et al.* (2008), total flavonoid concentrations ranged from 0 to 254 mg 100 g^{-1}, with around 75% of specimens containing flavonoids > 0.5 mg 100 g^{-1} and a collection average of 33 mg 100 g^{-1}.

Isolation of Candidal Isolates

Out of 50 oral swabs, 15 (40%) of the 38 *Candida* isolates were *C. albicans*, while 23 (60%) were non-*albicans*. Different authors have reported similar findings in the literature (Dharmeswari *et al.* 2014), with *C. albicans* being the most prevalent species in both vaginal and oral infections, with 45% and 47%, respectively. These findings support (Javad *et al.* 2015) that *C. albicans* and *C. glabrata* were the most commonly identified yeast species in patients.

Biofilm formation by *C. albicans*

All *C. albicans* were biofilm producers in this study, with varying strength values depending on the OD value and crystal violate. Low biofilm producers accounted for 7 (47%) of the total, while moderate and high biofilm producers accounted for 8 (53 %; Fig. 2). These findings indicate that BF is higher in cases of oral infection than in cases of vaginal infection. These findings are consistent with those of Imran & Alshammry (2016) who reported that 100% of *C. albicans* isolated from various sources could produce biofilm in vitro. It also agreed with (Mahmoudabadi *et al.* 2017) who discovered that all *C. albicans* isolates tested formed biofilm on polystyrene.

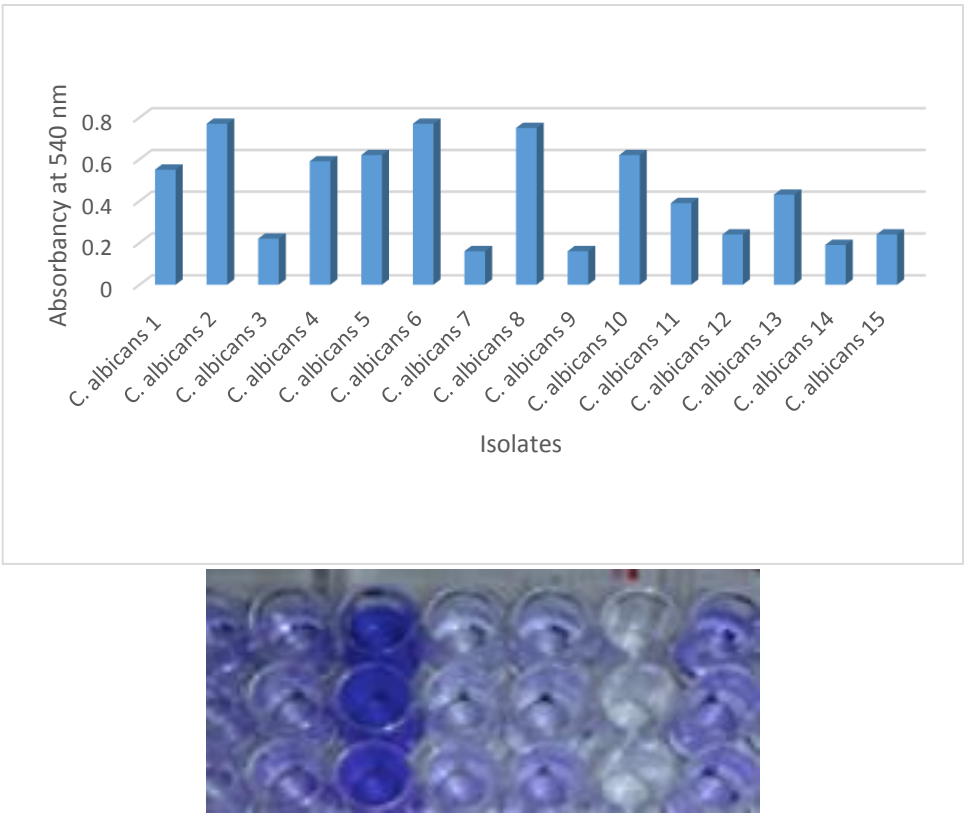

Fig. 2. Biofilm formation by *C. albicans* isolates.

Antibiofilm formation activity of flavonoids

Flavonoids isolated from the leaves and stems of the *C. spinosa* plant were found to have antibiofilm activity against all *C. albicans* isolates dependent on dose manner. In comparison to the control, flavonoids influence all *C. albicans*, at 400 µg mL^{-1}, with percentages of biofilm inhibition ranging from 69 to 31 % (Table 1). Apart from flavonoids, terpenes are one of the most potent chemical components in propolis, capable of damaging cell membranes and inhibiting *C. albicans* cell function (Prawiro *et al.* 2021). Because biomaterial infections are becoming an increasingly serious concern. These findings imply that flavonoids could be useful in treating biofilm-associated *C. albicans* infections.

Table 1. Detection of antifungal activity of flavonoids against *Candida albicans* isolates.

	Absorbency at 450 nm		
***Candida* spp.**	**After treatment with flavonoids**	**Before treatment with flavonoids**	**Inhibition of biofilm formation (%)**
C. albicans 1	0.17	0.55	69
C. albicans 2	0.33	0.77	57.1
C. albicans 3	0.10	0.22	54.5
C. albicans 4	0.21	0.59	64.4
C. albicans 5	0.19	0.62	69
C. albicans 6	0.33	0.77	57.1
C. albicans 7	0.12	0.16	29.4
C. albicans 8	0.31	0.75	58.6
C. albicans 9	0.11	0.16	31.2
C. albicans 10	0.22	0.62	64.5
C. albicans 11	0.19	0.39	51.2
C. albicans 12	0.13	0.24	45.8
C. albicans 13	0.20	0.43	53,4
C. albicans 14	0.11	0.19	42.1
C. albicans 15	0.13	0.24	45.8

CONCLUSION

Flavonoids could be useful in treating biofilm-associated *Candida albicans* infections to overcome the difficulties associated with multi-antibiotic resistance.

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