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Full Length Research Paper

Variability in antimicrobial activity of latex from two varieties of *Ficus carica*

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Fig latex has been a typical component in the health-promoting Mediterranean diet for millennia. To study its potential constituents, two varieties differing in color of fruit [Bidhi Bither: (BB); Kahli Bither: (KB)] and their growth area were analyzed for their organic extracts against sixteen microbes. The capacity of extracts was evaluated based on the inhibition zone, using the disc-diffusion assay, minimal inhibition concentration (MIC) for bacteria and yeasts, the method of calculating the inhibition percentage (I%) and Agar punched wells method for fungi, respectively. Extracts of (BB_1) variety from Chott Meriam showed the highest antimicrobial activities against the studied microorganisms when compared to (KB) variety and those from Mahdia. Ethyl acetate extracts had inhibition effect on the multiplication of *Enterococcus faecalis*, *Citobacter freundii*, *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Escherchia coli*. For the opportunist pathogenic yeasts, ethyl acetate and chloroformic fractions showed a very strong inhibition (100%) and an MIC of 0.082 and 1.25 µg/ml for (BB) and (KB), respectively, while ethanolic fraction had a total inhibition against *Candida albicans*, *Candida glabrata*, *Candida krusei* and *Candida parapsilosis*. *Microsporum canis* was strongly inhibited with ethanolic extract (75%) and totally with ethyl acetate extract at a concentration of 500 µg/ml.

Key words: *Ficus carica*, latex, organic extracts, fungi, yeasts, bacteria, antimicrobial activity.

INTRODUCTION

Mycosis and other diseases caused by microbes, such as bacteria and fungi, remain one of the serious health problems. Tunisian traditional system of medicine, like others in the world, has put forward a number of medicinal plants and their formulation for antibacterial and antifungal, such as candidose activities. In the modern age, it is very important to provide scientific proof to justify the various medicinal uses of herbs and plants or part of plants. *Ficus carica* L. (Moraceae) are used as constipation, hemorrhoid and high cholesterol in traditional medicine (Çakilcioğlu and Türkoğlu, 2007; Koyuncu et al., 2009; Cansaran and Kaya, 2010) and is consumed raw (uncooked) to treat constipation (Çakilcioğlu et al., 2010).

Herbal drugs are prescribed widely even when their biologically active compounds are known because of their effectiveness, fewer side effects and relatively low cost (Valiathan, 1998). However, we are not aware of a satisfactory remedy for serious microbial diseases and

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search for effective and safe drugs for this type of infections, which continue to be an area of interest.

F. carica is a deciduous tree, which grows in the Middle East and mainly around the Mediterranean basin (Sadder and Ateyyeh, 2006). Since ancient times, figs have been used for human consumption and recently, their nutritive and pharmacological values have been investigated. The consumption of figs helps and prevent vein blockage (Wang et al., 2003), and its high content in fibers has a laxative effect (Zouaoui, 1992). The previous studies reported the hypoglycemic action of fig leaves decoction in type-diabetic I patients (Teixeira et al., 2006). Recently, Canal et al. (2000) used a chloroform extract obtained also from a decoction of *F. carica* leaves to decrease the cholesterol level of diabetic rats (Teixeria et al., 2006). The pharmacological properties are probably in part due to the high content of enzymes, flavonoïdes and furanocoumarines from its latex (Chevallier, 2001).

The study's interest in *F. carica* species arose from the contrasting biological activities of its latex components. In this paper, the results were presented on the antimicrobial properties of the latex extract fractions using different organic solvents of two *F. carica* varieties grown in two different regions of Tunisia (Bidhi Bither have green fruit and Khali Bither have brown fruit).

The first samples are from Mahdia agriculture field harvested from two big trees that are more than one hundred years old and the second samples, some decades old, are from the High school of Horticulture of Chott Meriam from the same variety. The latex production of bifère variety depends on the season, in that the tree gives two different harvests per year, the first one being the most important called fig flowers and the second is called figs of the year.

MATERIALS AND METHODS

F. carica latex was collected from unripe fig fruit varieties: Bidhi Bither 1 (BB_1), Kahli Bither 1 (KB_1), Bidhi Bither 2 (BB_2) and Kahli Bither 2 (KB_2), grown in the botanical garden of Horticulture High School of Chott Meriam and in an agriculture field of fig trees of Mahdia, Southbound of the Tunisian middle coast (Sahel), respectively.

The fig fruit was cut open from its top then slightly squeezed to collect a few drops of latex in sterile haemolysed tubes, and then held in ice during gathering before it was stored at -30°C until further use. The fruit was carefully chosen between two states (youthful and ripe). The recognition of this variety was established following the preliminary observations, according to the botanical descriptions of the species, which is in the flora of Tunisia.

Extraction and isolation

The latex of *F. carica* varieties (BB_1, BB_2, KB_1 and KB_2) were separately lyophilized and 100 g of each latex variety was repeatedly macerated in 100% ethanol (Merck, Germany) during 3 days (Sarang et al., 2005) and the yellow ethanolic solutions were subsequently filtered and evaporated under reduced pressure to collect the remaining brown gummy residues.

The concentrated residues were separately portioned between hexane and aqueous ethanol. The hexane soluble was evaporated and the residues were subjected consecutively to silica gel chromatography, eluting it with hexane (Merck, Germany) [P_2 (KB_1), P_2 (KB_2), P_2 (BB_1), P_2 (BB_2)], through hexane-ethyl acetate (v/v) to ethyl acetate (Merck, Germany). In products 3: P_3 (KB_1), P_3 (KB_2), P_3 (BB_1) and P_3 (BB_2), each fraction was monitored by TLC, and the compounds migrated as a single band (Rf 0.7) on thin-layer chromatography (Shai et al., 2001). Resultantly, three crude fractions were obtained and evaporated under reduced pressure to give products 2: P_2 (KB_1), P_2 (KB_2), P_2 (BB_1) and P_2 (BB_2) and products 3: P_3 (KB_1), P_3 (KB_2), P_3 (BB_1) and P_3 (BB_2). Products 2 were white in color, while products 3 were yellow in color.

The remaining aqueous ethanolic layers were decanted with chloroform, to get chloroformic fractions which were also evaporated in a rotavapor to get green products: P_4 (KB_1), P_4 (KB_2), P_4 (BB_1) and P_4 (BB_2). The ethyl acetate fractions were found to contain two major spots for each variety by TLC (mobile phase: 5% ethyl acetate and 95% hexane, pulverized with sulphuric acid H_2SO_4) on silica gel 60 F_{254} plates (Merck, Germany).

Plates were visualized and detected under UV light (254 nm) (Mellou et al., 2005), before HPLC purity tests were determined (Table 1).

Microbial strains

Four products of lyophilized *F. carica* latex of each variety and areas were tested against ten strains of fungi comprising three dermatophytes (*Trichophyton rubrum*, *Trichophyton soudanense* and *Microsporum canis*), two hyphomycetes (*Aspergellus fumigates* and *Scopulariopsis brevicaulis*) and five opportunists pathogenic yeasts (*Candida albicans*, *Candida glabrata*, *Candida kreusei*, *Candida parapsilosis* and *Cryptococcus neoformans*). These microorganisms were obtained from Institute Pasteur, Paris France; Laboratoire de Microbiologie Faculté de Medecine, Besançon, France and Hôpital Universitaire Fattouma Bourguiba, Monastir, Tunisia.

Of these six tested bacteria, three were gram-positive: *Staphylococcus aureus* (ATCC25923), *Enterococcus faecalis* (ATCC29212) and *Citobacter freundii* (Clinical isolated), and three were gram-negative: *Pseudomonas aeruginosa* (ATCC27853), *Proteus mirabilis* (Clinical isolated) and *Escherchia coli* (ATCC25922). Bacteria were obtained from culture collection in the laboratory of transmissible diseases, Faculté de Pharmacie, Monastir, Tunisia.

Antimicrobial activity

Disc-diffusion assay

The dried latex extracts were dissolved in ethanol (10%) to a final dilution of 10 mg/ml (Mellou et al., 2005) and sterilized by filtration at 0.45 µm Millipore filters.

Antimicrobial tests were carried out by disc-diffusion method (Murray et al., 1995), using 100 µl of suspension containing 10^8 CFU/ml of bacteria and 10^6 CFU/ml of yeast, spread on Muller-Hinton Agar (MH-KAD) (Tanaka, 1992) and Sabouraud dextrose Agar (SDA) medium, respectively. The discs (6 mm in diameter) were impregnated with 10 µl of the extracts (100 µg/disc) at a concentration of 10 mg/ml and placed on the inoculated Agar.

Negative controls were prepared using 10% ethanol solvent employed to dissolve the latex extracts. Gentamicine (10 µg/disc) and nystatine (10 µg/disc) were used as positive reference standards to determine the sensitivity of one strain/isolate in each tested microbial species. The inoculated plates were incubated at

Table 1. The respective mass of extractions products.

Products	Varieties			
	BB_1 (g)	BB_2 (g)	KB_1 (g)	KB_2 (g)
P_1	24.00	27.00	17.00	26.00
P_2	4.10	6.30	2.20	5.00
P_3	4.00	4.50	2.40	3.00
P_4	2.70	3.00	1.70	2.90

BB_1: Bidhi Bither from Mahdia; BB_2: Bidhi Bither from Chott Meriam; KB_1: Kahli Bither from Mahdia; KB_2: Kahli Bither from Chott Meriam; P_1: Ethanolic fraction; P_2: Hexanoic fraction; P_3: Ethyl acetate fraction; P_4: Chloroformic fraction.

37°C during 24 h for bacterial strains and 48 h for yeasts. Antimicrobial activity was evaluated by measuring the inhibition zone against the tested microorganisms. However, each assay was duplicated.

Micro dilution assay

The minimal inhibitory concentration (MIC) values were also studied for bacteria and yeasts strains were determined as sensitive to extracts (P_1, P_2, P_3 and P_4) for each variety in disc-diffusion assay. The inoculums of microorganisms were prepared from 12 h broth cultures and suspensions were adjusted to 0.5 Mc Farland standard turbidity. Extracts dissolved in 10% ethanol were first diluted to the highest concentration (500 µg/ml), and then serial two-fold dilutions were made in a concentration range from 7.8 to 500 µg/ml in 10 ml sterile test tubes containing Muller-Hinton Broth (MHB). MIC values of extracts against bacterial strains and yeast isolate were determined based on a micro-well dilution method (Zgoda and Porter, 2001) and described with some modifications.

The 96-well plates were prepared by dispensing 95 µl of nutrient broth and 5 µl of the inoculum into each well. Subsequently, 100 µl from each extract initially prepared at the concentrations of 500 µg/ml were added into the first wells of different plates (one plate for each extract). Then 100 µl from their serial dilutions were transferred into six consecutive wells. The last well of each plate containing 195 µl of MHB without compound and 5 µl of the inoculum on each strip was used as negative control. The final volume in each well was 200 µl. Gentamicine (MEGENTAL) and Nystatine (for bacteria and yeasts, respectively) at the concentration range of 500 to 7.8 µg/ml, were prepared in the nutrient broth and used as a standard drug for positive control. The plate was covered with a sterile plate sealer. The content of each well was mixed on a plate shaker at 300 rpm for 29 s and then incubated at an appropriate temperature for 24 h. Microbial growth was examined by binocular microscope and was confirmed by plating 5 µl samples from clear wells on NA medium. The extracts tested in this study were screened twice against each microorganism. The MIC was defined as the lowest concentration of the compound to inhibit the growth of microorganisms. Confirmations were determined when 5 µl samples from clear well plotting onto NA plate without extracts had no microbial growth.

Agar incorporation method

The antifungal activities of fractions were assayed using Agar incorporation dilution method in a solid medium (Eloff, 1998) including negative control as previously described by Bel Haj Salah et al. (2007).

The fractions were aseptically mixed with 100 ml of Sabouraud Glucose Agar (SGA) giving a final concentration of 500 µg/ml. All fractions were dissolved in 99% ethanol and were used as a negative control (Zgoda and Porter, 2001). After cooling and solidification of SGA in sterile Petri dishes (33 mm in diameter), the medium was inoculated with a small amount (5 mm in diameter) of a seven-days-old mycelium culture of tested dermatophytes and hyphomycetes and three-days-old yeast culture suspended in sterile distilled water and adjusted to 10^5 spores/ml of opportunist pathogenic yeasts (*C. albicans*, *C. neoformans*, *C. glabrata*, *C. kreussei* and *C. parapsilosis*) (Giordani et al., 2001). The Petri dishes were then incubated at 24°C during seven days for dermatophytes and *Scopulariopsis* (hyphomycete) at 37°C during 24 h for *C. albicans*, *C. glabrata*, *C. kreussei*, *C. parapsilosis*, and *A. fumigatus*, during 48 h for *C. neoformans*. All tests were carried out in triplicate.

The antifungal activity of the extracts was evaluated using the method by calculating the inhibition percentage (I%) from the colonies added to the assayed extracts (dE), I% = (dC - dE)/dC, as described by Pandey et al. (1982) and Tegegne et al. (2008).

Antifungal assay using wells punched method

An agar disc (6 mm in diameter) with *T. rubrum*, *T. soudanense*, *M. canis*, *A. fumigatus* and *S. brevicaulis* was derived from the fungi in an actively growing state previously and was cultured on a Sabouraud Glucose Agar (SGA), whose strain was placed in the centre of a Petri dish containing SGA (Toki et al., 2005).

The plates were incubated at room temperature for 12 h. Wells were subsequently punched into the Agar at a distance of 15 mm from the centre of the Petri dish. The samples to be tested were placed in to the wells in 10 µl of sterile water. The plates were incubated during 24 h at a room temperature and then the distances of the inhibition zone around the wells were measured.

RESULTS

In the present study, the antimicrobial compounds from *F. carica* latex extracts collected from two different areas of the Sahel region of Tunisia were tested against a considerable range of microorganisms on the basis of disc-diffusion, well punched Agar incorporation and micro-dilution assay.

Antifungal activities

The antifungal activities of extracts against microorganisms examined and their potency were quantitatively assessed by the presence or absence of inhibition zones around the punched wells and by 1% values shown, respectively in Tables 2 and 3.

Table 2. Antimicrobial activity of extracts (100 µl/well) against tested fungi strains based on punched well method.

Fungi	[a] Inhibition zone diameter in mm																N
	P_1				P_2				P_3				P_4				
	BB_1	BB_2	KB_1	KB_2	BB_1	BB_2	KB_1	KB_2	BB_1	BB_2	KB_1	KB_2	BB_1	BB_2	KB_1	KB_2	
T. ruburum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	17
T. soudanense	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	16
M. canis	7-10	7-12	7-9	7-11	7-10	7-12	7-9	7-11	15-20	15-22	13-17	13-20	-	-	-	-	18
A. fumigatus	-	-	-	-	-	-	-	-	7-22	7-24	7-16	7-18	7-15	7-16	7-14	7-15	18
S. brevicaulis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	19

[a]: average of the zone diameters of inhibition in mm including well diameter of 6 mm; N: Nystatine; BB_1: Bidhi Bither from Mahdia; BB_2: Bidhi Bither from Chott Meriam; KB_1: Kahli Bither from Mahdia; KB_2: Kahli Bither from Chott Meriam; P_1: Ethanolic fraction; P_2: Hexanoic fraction; P_3: Ethyl acetate fraction; P_4: Chloroformic fraction.

Table 3. Antifungal activity of extracts (500 µg/ml) using percentage inhibition of microorganisms.

Fungi	[p] Inhibition zone %															
	P_1%				P_2%				P_3%				P_4%			
	BB_1	BB_2	KB_1	KB_2	BB_1	BB_2	KB_1	KB_2	BB_1	BB_2	KB_1	KB_2	BB_1	BB_2	KB_1	KB_2
T. ruburum	10	20	10	20	16	20	10	20	10	60	10	50	10	30	10	30
T. soudanense	10	10	10	10	10	10	10	10	10	30	10	20	0	0	10	20
M. canis	57	75	40	50	57	76	50	75	86	100	60	90	28	42	10	30
A. fumigatus	20	30	10	20	30	40	20	30	80	100	70	75	40	50	20	30
S. brevicaulis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

[P] 0-20%, no or little inhibition; 21-50%, average inhibition; 51-100%, strong inhibition; BB_1: Bidhi Bither from Mahdia; BB_2: Bidhi Bither from Chott Meriam; KB_1: Kahli Bither from Mahdia; KB_2: Kahli Bither from Chott Meriam; P_1: Ethanolic fraction; P_2: Hexanoic fraction; P_3: Ethyl acetate fraction; P_4: Chloroformic fraction.

The results showed that *M. canis* was inhibited by fractions (P_1), (P_2) and (P_3), but (P_4) (chloroformic fractions) had no effect on this strain. The inhibition diameter around the wells varied from 7 to 22 mm; the highest result obtained against this germ was shown with (P_3) (ethyl acetate extracts) from 15 to 22 mm and was totally inhibited (I=100%) at a concentration of 500 µg/ml with the second method (dilution in a solid medium) (Table 3).

A. fumigatus was more sensitive against ethyl acetate extracts than the chloroformic ones. *T. rubrum*, *T. soudanense* and *S. Brevicaulis* were resistant against all tested extracts. In this study, the dermatophytes were more resistant to these extracts except *M. canis* filamentous fungi which were sensitive against ethyl acetate fraction.

Extracts activities against yeasts

The results of the tested yeasts (Tables 4, 5 and 6) showed that ethyl acetate extracts were most active against the studied yeasts. *C. neoformans*, *C. glabrata* and *C. albicans*, were the most sensitive strains against latex extracts, although diameter of inhibitions varied from 15 to 28 mm by disc-diffusion assay method and were totally inhibited (100% = I) by dilution in the solid medium. Therefore, the MIC against these strains was about 0.041 µg/ml and it confirmed that *F. carica* latex can be an efficient anticandidal drug.

Antibacterial activities

The antibacterial activity of latex extracts as

Table 4. Antimicrobial activity of extracts (100 µg/disc) against tested yeasts strains based on disc-diffusion method.

Yeasts	[b] Inhibition zone diameter in mm																N
	P_1				P_2				P_3				P_4				
	BB_1	BB_2	KB_1	KB_2	BB_1	BB_2	KB_1	KB_2	BB_1	BB_2	KB_1	KB_2	BB_1	BB_2	KB_1	KB_2	
C. albicans	15-28	15-26	11-22	15-24	8	8	7	7	15-28	15-26	11-22	13-24	26	28	23	25	17
C. glabrata	18	16	14	12	-	-	-	-	7-15	7-17	7-14	7-15	7-16	7-17	7-13	7-14	21
C. kreussei	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	23
C. parapsilosis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	22
C. neoformans	-	-	-	-	7	9	7	8	15-26	15-28	12-22	12-24	11-24	11-26	7-14	7-15	26

N: Nystatine; [b]: average of the zone diameters of inhibition in mm including disk diameter of 6 mm; BB_1: Bidhi Bither from Mahdia; BB_2: Bidhi Bither from Chott Meriam; KB_1: Kahli Bither from Mahdia; KB_2: Kahli Bither from Chott Meriam; P_1: Ethanolic fraction; P_2: Hexanoic fraction; P_3: Ethyl acetate fraction; P_4: Chloroformic fraction.

Table 5. Antimicrobial activity of extracts (500 µg/ml) against tested yeasts strains based on Agar incorporation method.

Yeasts	[P] Inhibition zone %															
	P_1%				P_2%				P_3%				P_4%			
	BB_1	BB_2	KB_1	KB_2	BB_1	BB_2	KB_1	KB_2	BB_1	BB_2	KB_1	KB_2	BB_1	BB_2	KB_1	KB_2
C. albicans	100	100	80	90	20	25	10	25	90	100	70	90	100	96	100	96
C. glabrata	40	45	10	12	0	0	0	0	10	20	10	15	100	96	100	96
C. kreussei	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C. parapsilosis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C. neoformans	-	-	-	-	25	30	10	20	90	100	80	96	96	100	80	96

[P] 0-20%, no or little inhibition; 21-50%, average inhibition; 51-100%, strong inhibition; BB_1: Bidhi Bither from Mahdia; BB_2: Bidhi Bither from Chott Meriam; KB_1: Kahli Bither from Mahdia; KB_2: Kahli Bither from Chott Meriam; P_1: Ethanolic fraction; P_2: Hexanoic fraction; P_3: Ethyl acetate fraction; P_4: Chloroformic fraction.

Table 6. The minimum inhibitory concentration (MIC) of *Ficus carica* latex extracts against yeasts.

Yeasts	MIC values of latex extracts against yeasts - Minimum inhibitory concentration (mg/ml)																N
	P_1				P_2				P_3				P_4				
	BB_1	BB_2	KB_1	KB_2	BB_1	BB_2	KB_1	KB_2	BB_1	BB_2	KB_1	KB_2	BB_1	BB_2	KB_1	KB_2	
C. albicans	0.082	0.141	0.082	0.082	2.50	2.50	2.50	2.50	0.082	0.041	0.082	0.082	0.082	0.041	0.082	0.041	0.33
C. glabrata	1.25	1.25	1.25	1.25	-	-	-	-	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	0.33
C. kreussei	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.33
C. parapsilosis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.25
C. neoformans	-	-	-	-	2.50	2.50	2.50	2.50	0.082	0.041	0.082	0.041	0.125	0.82	0.125	0.82	0.66

N: Nystatine (mg/ml); BB_1: Bidhi Bither from Mahdia; BB_2: Bidhi Bither from Chott Meriam; KB_1: Kahli Bither from Mahdia; KB_2: Kahli Bither from Chott Meriam; P_1: Ethanolic fraction; P_2: Hexanoic fraction; P_3: Ethyl acetate fraction; P_4: Chloroformic fraction.

Table 7. Antimicrobial activity of extracts (100 µg/disc) against tested bacterial strains based on disc-diffusion method.

Bacteria	Inhibition zone diameter in mm																
	P_1				P_2				P_3				P_4				G
	BB_1	BB_2	KB_1	KB_2	BB_1	BB_2	KB_1	KB_2	BB_1	BB_2	KB_1	KB_2	BB_1	BB_2	KB_1	KB_2	
S. aerus	-	-	-	-	8-12	12	8-11	11-12	12	13	8-9	8-11	13	14	8-9	9-11	12-24
E. faecalis	-	-	-	-	11	14	11	13	8	8-10	7-9	7	8	9-12	7	7-10	20-25
C. freundii	11	14	9	12	12	14	9	12	14	16	14	12	14	14-16	12	12-14	-
P. aeruginosa	9	14	7	12	9-12	8-12	8-10	7-11	8-10	9-12	7-10	6-7	8-9	9-12	6-7	7-9	14-16
P. mirabilis	13	16	12	14	12	15	11	14	15	16	15	12	13	16	12	15	8
E. coli	10	12	9	11	10	15	9	13	12	15	14	9-10	10	14	9-10	9-14	10-17

G, Gentamicine (mg/ml); BB_1: Bidhi Bither from Mahdia; BB_2: Bidhi Bither from Chott Meriam; KB_1: Kahli Bither from Mahdia; KB_2: Kahli Bither from Chott Meriam; P_1: Ethanolic fraction; P_2: Hexanoic fraction; P_3: Ethyl acetate fraction; P_4: Chloroformic fraction.

Table 8. Minimum inhibitory concentration (MIC) values of extracts against bacteria.

Bacteria	MIC values of latex extracts against bacteria - Minimum inhibitory concentration (mg/ml)																
	P_1				P_2				P_3				P_4				G
	BB_1	BB_2	KB_1	KB_2	BB_1	BB_2	KB_1	KB_2	BB_1	BB_2	KB_1	KB_2	BB_1	BB_2	KB_1	KB_2	
S. aerus	0.66	0.33	0.66	0.66	0.66	0.33	0.66	0.66	2.50	1.25	2.50	1.25	5.00	5.00	5.00	5.00	0.33
E. faecalis	2.50	1.25	2.50	1.25	1.25	0.66	1.25	1.25	2.5	1.25	2.50	2.50	2.50	2.50	2.50	2.50	0.33
C. freundii	1.25	0.66	1.25	0.66	2.50	1.25	2.50	2.50	0.66	0.33	0.66	0.33	0.66	0.33	0.66	0.66	0.33
P. aeruginosa	-	-	-	-	5.00	5.00	5.00	5.00	-	-	-	-	-	-	-	-	0.33
P. mirabilis	0.33	0.165	0.33	0.165	0.33	0.165	0.33	0.165	0.08	0.041	0.08	0.08	0.08	0.041	0.08	0.041	1.125
E. coli	1.25	0.66	1.25	0.66	1.25	0.66	1.25	0.66	5.00	5.00	5.00	5.00	1.25	0.66	1.25	0.66	0.66

G, Gentamicine (mg/ml); BB_1: Bidhi Bither from Mahdia; BB_2: Bidhi Bither from Chott Meriam; KB_1: Kahli Bither from Mahdia; KB_2: Kahli Bither from Chott Meriam; P_1: Ethanolic fraction; P_2: Hexanoic fraction; P_3: Ethyl acetate fraction; P_4: Chloroformic fraction.

shown in Table 7 was examined by the presence or absence of inhibition zone diameter. These results showed that the ethyl acetate extracts had inhibition effect on the growth of five bacterial species: *E. faecalis*, *C. freundii*, *P. aeruginosa*, *E. coli* and *P. mirabilis*. The inhibition values on these microorganisms were sensitive to ethyl acetate extracts in the range of 8 to 16 mm, while hexanoic and chloroformic extracts were active against these six tested bacteria at a sensitive range of 8 to 15 and 8 to 14 mm, respectively. Methanolic extracts had no effect against the previous bacteria except for *P. mirabilis* with inhibition diameter of 14 mm (Bidhi Bither: BB_1, Chott Meriam variety).

The antimicrobial activity of extracts with micro-well dilution assay was shown in Table 8. All extracts exhibited an antibacterial activity against selected microorganisms at different levels. *P. mirabilis* was the most sensitive germ at a range of 0.33 to 0.041 mg/ml. The Hexanoic extracts were the only fractions active against *P. aeruginosa* which was the most resistant germ at MIC of 5.00 mg/ml. These extracts exhibited the most important activity against *P. mirabilis* and *S. auerus*. *E. coli* was also inhibited by all fractions at concentrations of 5 to 0.66 mg/ml.

DISCUSSION

The study's data showed on the one hand that there was no uniform response between bacterial and fungal strains. In terms of susceptibility to antimicrobial compounds in different extracts of the studied varieties, extracts from Bidhi Bither latex were more active than Kahli Bither. On the other hand, extracts of latex from varieties cultivated in Chott Merian were more active than those from Mahdia. We have to mention that the latex collected from Mahdia was from an agricultural field of two cultivated varieties, while nine different varieties of *F. carica* plants comprising four varieties of caprifig (Dokhar: male sex tree) were cultivated from the Botanical Garden of Horticulture High School, Chott Meriam. Indeed, the existence of different varieties increases the medicinal effect of plants while the selection decreases the biological potential.

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