

# IN VITRO ANTIMICROBIAL ACTIVITY OF FOUR *FICUS CARICA* LATEX FRACTIONS AGAINST RESISTANT HUMAN PATHOGENS (ANTIMICROBIAL ACTIVITY OF *FICUS CARICA* LATEX)

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## ABSTRACT

Methanolic, hexanoïc, chloroformic and ethyl acetate extracts of *Ficus carica* latex were investigated for their in vitro antimicrobial proprieties against five bacteria species and seven strains of fungi. The green fruit latex was collected from Chott Mariam Souse, Middle East coast of Tunisia. The antimicrobial activity of the extracts was evaluated and based respectively on the inhibition zone using the disc – diffusion assay, minimal inhibition concentration (MIC) for bacterial testing and the method by calculating inhibition percentage (I%) for fungi-inhibiting activities.

The methanolic extract had no effect against bacteria except for *Proteus mirabilis* while the ethyl acetate extract had inhibition effect on the multiplication of five bacteria species (*Enterococcus fecalis*, *Citobacter freundei*, *Pseudomonas aeruginosa*, *Echerchia coli* and *Proteus mirabilis*). For the opportunist pathogenic yeasts, ethyl acetate and chlorophormic fractions showed a very strong inhibition (100%); methanolic fraction had a total inhibition against *Candida albicans* (100%) at a concentration of 500µg/ml and a negative effect against *Cryptococcus neoformans*. *Microsporium canis* was strongly inhibited with methanolic extract (75%) and totally with ethyl acetate extract at a concentration of 750µg/ml. Hexanoïc extract showed medium results.

**Keywords:** Antibacterial activity; antifungal activity; dermatophytes; *Ficus carica*; latex; organic extracts.

## INTRODUCTION

The fig tree (*Ficus carica L.*) is a typical Mediterranean species and probably the first intentionally grown plant during the Neolithic revolution. Its domestication preceded that of cereals by about a thousand years (Kislev *et al.* 2006). Fig trees have thrived in the Middle East and mainly around the Mediterranean basin (Sadder *et al.*, 2006), their syconias have provided valuable food for people and animals (Bandelj *et al.*, 2007). 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 prevent vein blockage (Wang *et al.*, 2003) and its high content in fibers has laxative effects (Zouaoui, 1992). The previous studies reported the hypoglycemic action of fig leaves decoction in type – diabetic I patients (Martins Teixeira *et al.*, 2006), more recently in 2000, Lanal used a chloroform extract, obtained also from a decoction of *F. carica* leaves, to decrease the cholesterol levels of diabetic rats (Martins Teixeira *et al.*, 2006).

The pharmacological properties are probably in part due

to the high content of phenolic compounds in these plant extracts. Some phenolic compounds with reported pharmacological proprieties have already been isolated from fig leaves, namely funarocoumarins like psoralen and bergapten (Martins Teixeira *et al.*, 2006), flavonoïds like rutin, phenolic acids like feirulic acids, and also phytosterols like taraxasterol (Martins Teixeira *et al.*, 2006). Vitamin B<sub>1</sub> and B<sub>2</sub> are also found in figs (Omar *et al.*, 2004). D- glucans have been found as common polymers of the fungal cell wall, they consist of a mixture of linear (1 → 6) – β – D – glucan with various (1→6) – branched oligosaccharides chains, the length of these chains being modulated by culture conditions (Omar *et al.*, 2004).

Despite the fact that other parts of fig tree, like latex, have also been reported to have little known pharmacological properties; it inhibits the growth of carcinoma cells (Martins Teixeira *et al.*, 2006) and in traditional medicine it has been used to treat eyesore (Mars, 2003).

Our interest in *Ficus carica* species arose from the contrasting biological activities of its latex components.

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In this paper, we present the results of our study on the antimicrobial properties of fractions using different organic solvents.

## MATERIALS AND METHODS

### *Plant material*

*F. carica* latex was collected from fig unripe fruit growing in the botanical garden of Horticulture high school of Chott Mariem Sousse Tunisia. This fig fruit was cut open from its top then slightly squeezed to collect a few drops of latex in a sterile tube then stored at (-30°C) until further use.

### *Extraction and isolation*

Latex of *F. carica* was lyophilized and 100g of powdered latex was repeatedly macerated in methanol 100% (Merck, Germany) during 3 days (Sarang *et al.*, 2005) and the yellow methanolic solution was subsequently filtered and evaporated under reduced pressure to collect the remaining brown gummy residue (18g) Product1(P<sub>1</sub>).

The concentrated residue was portioned between hexane and aqueous methanol, the hexane soluble were evaporated and the residue was subjected to silica gel chromatography eluting with hexane (Merck, Germany), product 2 (P<sub>2</sub>), through hexane-ethyl acetate (50: 50) to ethyl acetate (Merck, Germany), product 3 (P<sub>3</sub>), each fraction was monitored by TLC, the compounds migrated as a single band (R<sub>f</sub> 0,7) on thin-layer chromatography (Shai, R. *et al.* 2001) three crude fractions were obtained and evaporated under reduced pressure to give product 2 (P<sub>2</sub>) and 3 (P<sub>3</sub>). Product 2 (2,30g) was white and product 3 (2,40g) was yellow. The remaining aqueous methanolic layer was decanted with chloroform, to get chloroformic fraction which was also evaporated in a Rotavapor to get a green product 4 (1,70g) (P<sub>4</sub>), the ethyl acetate fraction was found to contain two major spots by .T.LC (mobile phase; 5% ethyl acetate, 95% hexane, pulverized with sulfuric acid H<sub>2</sub>SO<sub>4</sub>) on silica gel 60 F<sub>254</sub> plates (Merck, Germany). Plates were visualized and detected under UV light (254nm) (Mellou *et al.*, 2005), HPLC purity was determined by HPLC tests.

### *Microbial strains*

The four products (P<sub>1</sub>, P<sub>2</sub>, P<sub>3</sub> and P<sub>4</sub>) of lyophilized *F. carica* latex were tested against seven strains of fungi comprising three dermatophytes (*Trichophyton rubrum*, *Trichophyton soudanense*, *Microsporum canis*), two hyphamycets (*Aspergillus fumigatus*, *Scopulariopsis brevicaulis*) and two opportunists pathogenic yeasts (*Candida albicans* and *Cryptococcus neoformans*).

The microorganisms had two origins, Institute Pasteur, Paris and the laboratory of microbiology, faculté de médecine, Besançon, France.

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

### *Antifungal testing*

The antifungal activities of fractions were assayed with the method of agar incorporation "dilution in a solid medium" (Eloff, 1998) including negative control as previously described (Bel Hadj Salah *et al.*, 2007).

The fractions were aseptically mixed with 100ml of Saborogluucose Agar (SGA) giving a final concentrations of 500µg/ml, 750µg/ml.

All fractions were dissolved in ethanol 99% and this solvent was used as a negative control (Zgoda *et al.*, 2001). After cooling and solidification of (SGA) in sterile Petri dishes (33mm diameter), the medium was inoculated with a small amount (5mm diameter) of a seven-day-old mycelium culture of tested dermatophytes and hyphamycets, and a three-day-yeast culture suspended in sterile distilled water and adjusted to 10<sup>5</sup> spores/ml of opportunist pathogenic yeasts (*C. albicans* and *C. neoformans*) (Giordani *et al.*, 2001). The Petri dishes were then incubated at 24°C for seven days for dermatophytes and scopulariopsis (hyphamycete), 24 hours at 37°C for *Candida* and *A. fumegatus* and 48 hours at 37°C for *C. neoformans*. Three replicate for each concentration and microorganisms were carried out.

The antifungal activity of the extracts was evaluated using the method by calculating the inhibition percentage (I%) from the colony diameter values in the control plate (dC) and the colonies in the plate added to the assayed extracts (dE), I% = (dC - dE) / dC, according to Singh *et al.* method (Singh *et al.*, 1993).

### *Antibacterial activity*

#### *Paper disk diffusion assay*

Each sample was dissolved at 10mg/ml with ethanol 10% (Mellou *et al.*, 2005). Simultaneously, chlorophormic extract was dissolved in acetone 10% and hexanoic extract was dissolved in dimethyl sulfoxide 10% (DMSO; Merck, Germany), methanolic extract was dissolved in DMSO 10% and distilled water at 10mg/ml for each samples (Luilma, A.G. *et al.* 2005). A negative control disc was impregnated with 10µl of different solvents used in each experiment. The antibacterial activity was evaluated against three gram-positive bacteria:

*S. aureus* (ATCC25923) *E. fecalis* (ATCC29212) and *C. freundii* (clinical isolated), then three gram-negative bacteria: *P. aeruginosa* (ATCC27853), *P. mirabilis* (clinical isolated), *E. coli* (ATCC25922).

Organisms were maintained on Muller-Hinton agar (MH-RAD) (Tanaka, 1992).

The inoculums were prepared overnight (24h at 37° C) cultures in Muller Hinton broth medium (MHB). Petri dishes were inoculated with suspensions of 10<sup>6</sup> germs/ml (Rio et al., 1988).

Absorbent disks (Watman n° 3.5mm of diameter) were impregnated with 10µl of solution then placed on the surface of inoculated plates (90mm). Positive control discs of Gentamicine 10µl/ml were included in each essay. Diameters of growth inhibition zone were measured after incubation at 37° C for 24h (Barry et al., 1979). All experiments were performed in triplicate.

Other tests were made on the same germs in which we dissolved chloroformic extract in acetone 10%, hexanoic extract in dimethyl sulfoxide (DMSO) 10%, methanolic extract in DMSO 10% and distilled water at 10mg/ml for each samples (Luilma, A.G. et al. 2005). A negative control disc was impregnated with 10µl of different solvents used in each experiment

#### Micro-Well dilution essay

Minimum inhibitory concentrations (MIC) were performed by a serial dilution technique using 96 Well microliter plates (Mitscher et al., 1972). Bacterial species were cultured from 12-hour broth cultures and suspensions were adjusted to 0.5 McF arland standard turbidity (Bassole et al., 2003). All tests were performed in Muller Hinton broth (MHB). The fractions were first diluted in ethanol 10% to the highest concentration 10mg/ml. Serial two-fold dilutions were made in the eight consecutive wells in a concentration ranging from 500µg/ml to 40µg. 45µl of MHB and 5µl of the inoculums were then added in each well containing 50µl of compound. The last well containing 95µl of MHB without compound and 5µl of the inoculums on each strip was used as negative control. The final volume in each well was 100µl. Plates were incubated for 24 hours at 37°C then examined by a binocular microscope. The MIC was defined as the lowest concentration of the compounds to inhibit the growth of micro-organisms (Karaman et al., 2003). Ethanol (10%) was used as a negative control and Gentamicine (MEGENTAL) was used as positive control (Bel Hadj Salah et al., 2005). Minimum inhibitory concentrations of Gentamicine were also determined in parallel experiments at concentration range of 1mg to 7-8µg/ml. The tested extract in this study was screened in three replicates against each organism.

## RESULTS

### Antifungal activity

The antifungal activity of these four fractions was presented in table 1 and their inhibition varied from 0 to 100%.

For the opportunist pathogenic yeasts, ethyl acetate and chloroformic fractions showed a total inhibition of (100%). The methanolic fraction had a total effect against *C. albicans* (100%) and no effect on *C. neoformans* (0%).

*M. canis* was strongly inhibited with methanolic and hexanoic extracts (75%; 76%) and totally with ethyl acetate fraction (100%).

*A. fumigatus* was totally inhibited with ethyl acetate fraction (100%) while the hexanoic fraction had a parcel inhibition against tested germs.

*T. rubrum*, *T. soudanense* and *S. brevicans* were the most resistant germs against these four fractions.

### Antibacterial activity

The antibacterial activity of latex extracts are presented in table 2

The antibacterial activity of extracts dissolved in different solvents as shown in table 2 was examined by the presence or absence of inhibition zone diameters. These results showed that the ethyl acetate extract had inhibition effect on the growth of five bacterial species:

*E. fecalis*, *C. freundii*, *P. aeruginosa*, *E. coli* and *P. mirabilis*. The inhibition values on these microorganisms sensitive to the ethyl acetate extract were in the range of 10 to13mm, while hexanoic and chloroformic extracts were active against these six tested bacteria with a sensitive range of 7 to11mm and 6 to12mm respectively. Methanolic extract had no effect against the previous bacteria except for *P. mirabilis* with a diameter inhibition of 12mm.

The antibacterial activity of extracts with Micro-well dilution assay was present in table 3. All extracts exhibited an antibacterial activity against selected microorganisms at different levels. *P. mirabilis* was the most sensitive germ with a range of 0.33mg/ml to 0.08mg/ml. The hexanoic extract was the only fraction active against *P. aeruginosa* which was the most resistant germ with MIC of 5mg/ml. These extracts exhibited the most important activity against *P. mirabilis* and *S. aureus*. *E. coli* was also inhibited with all tested fractions at concentrations of 5mg/ml to 1.25mg/ml.

## DISCUSSION

In this study, we demonstrate that ethyl acetate latex extract contains substances which have antifungal, antibacterial and anticandidal effects, in other studies Sarang *et al.* in 2005, demonstrated that ethyl acetate fraction of the latex of *Euphorbia royleana* had immunosuppressive properties (Sarang *et al.*, 2005).

Chloroformic fraction had also important results compared to the other fractions.

These results confirmed the evidence that ethyl acetate and chloroform were the best solvents for many constituent extraction of latex antimicrobial substances compared to other solvents such as water, hexane, methanol etc.

Water and methanol are the most polar solvents that elute polar substances, hexane is a polar solvent that elutes non polar substances, but ethyl acetate and chloroform have a medium polarity, so the substances that have the most important biological activities were the medium polar ones.

Ahmad *et al.* (2001) demonstrated that only glycosides and saponin extracted from *F. carica* leaves using alcohol as solvent had biological effects but they had no effects on *C. albicans*, *S. aureus* and *E. coli* (Ahmad *et al.*, 2001), compared to our study latex extracts are more active than leaf extracts on human pathogenic yeasts and bacteria.

Our data showed that there was no uniform response between bacterial and fungal strains, in terms of susceptibility to antimicrobial compounds in different extracts (tables 1, 2, 3). These kinds of differences in susceptibility among microorganisms against antimicrobial substances in *F. carica* latex extracts may be explained by the differences in cell wall composition and or inheritance genes on plasmids that can be easily transferred among bacterial strains.

These results may suggest that ethyl acetate and chloroformic extracts of *F. carica* latex possess compounds with antibacterial and anticandidal properties which can be used as antimicrobial agents in new drugs for therapy of infections diseases. Thus, these compounds may find effective application for the treatment of

**Table 1:** Antifungal activity of the four *F. carica* latex extracts: Percentage of inhibition

Microorganisms	P <sub>1</sub>		P <sub>2</sub>		P <sub>3</sub>		P <sub>4</sub>	
	I <sub>1</sub> %	I <sub>2</sub> %	I <sub>1</sub> %	I <sub>2</sub> %	I <sub>1</sub> %	I <sub>2</sub> %	I <sub>1</sub> %	I <sub>2</sub> %
<i>Aspergillus fumigatus</i> (B)	20	20	30	-	80	100	50	50
<i>Trichophyton rubrum</i> (B)	10	10	16	20	10	60	10	30
<i>Trichophyton soudanense</i> (B)	10	10	10	10	10	30	00	10
<i>Microsporum canis</i> (IP)	57	75	57	76	86	100	28	42
<i>Scopulariopsis brevicaulis</i> (B)	10	10	00	00	00	50	00	20
<i>Candida albicans</i> (B)	100	100	25	25	100	100	96	96
<i>Cryptococcus neoformans</i> (B)	00	00	25	25	100	100	100	100

I<sub>1</sub>: percentage inhibition of microorganisms in the presences of (500 µg/ml) of extract;

I<sub>2</sub>: percentage inhibition of microorganisms in the presences of (750 µg/ml) of extract;

B: Microbiological laboratory, besançon France; IP: Institut Pasteur, Paris;

P<sub>1</sub>: methanolic fraction; P<sub>2</sub>: hexanoic fraction; P<sub>3</sub>: ethyl acetate fraction;

P<sub>4</sub>: chloroformic fraction.

**Table 2:** *In vitro* antibacterial activity of *F. carica* latex extracts in agar diffusion assay

Products	Growth inhibition zone diameter <sup>a</sup> (mm)						Gentamicine (10µg/ml)
	P <sub>1</sub>	P <sub>2</sub>		P <sub>3</sub>	P <sub>4</sub>		
	EtoH	DMSO	EtoH	EtoH	Acetone	EtoH	
<b>Bacteria</b>							
<i>Staphylococcus aureus</i> ATCC	-	9-11	7-8		8-9	6	13-25
<i>Enterococcus Fecalis</i> ATCC	-	11	7-9	10-12	-	7	20-25
<i>Citobacter freundei</i>	8	-	7	12	-	12	-
<i>Pseudomonas aeruginosa</i> ATCC	6	7-9	7-10	11-12	-	6-7	15-17
<i>Proteus mirabilis</i>	12	-	11	13	-	12	6
<i>Echerchia coli</i> ATCC	7	9	9	11	-	9-10	12-18

<sup>a</sup>Inhibition zones including the diameter of the paper disc (5mm); (-) no activity.

**Table 3:** The minimum inhibitory concentration (MIC) of *F. carica* latex extracts

The MIC values of latex extracts against tested bacteria Minimum inhibitory concentration (mg/ml)					
Microorganisms	Products				Gentamicine
	P <sub>1</sub>	P <sub>2</sub>	P <sub>3</sub>	P <sub>4</sub>	
<i>Staphylococcus aerus</i> ATCC	0.66	0.66	2.50	5.00	0.33
<i>Euterococcus felicalis</i> ATCC	2.50	1.25	2.50	2.50	0.33
<i>Citobacterfreundei</i>	1.25	2.50	0.66	0.66	0.33
<i>Pseudomonas aeruginosa</i> ATCC	-	5.00	-	-	0.33
<i>Proteus mirabilis</i>	0.33	0.33	0.08	0.08	1.25
<i>Echerchia coli</i> ATCC	1.25	1.25	5.00	1.25	0.66

MIC: minimum inhibitory concentration (mg/ml); (-): no activity.

superficial fungal and bacterial infections, particularly in cutaneous mycosis. In fact, the present finding justifies the use of *F. carica* latex in traditional medicine for the treatment of various disease conditions whose symptoms might involve fungal infections and points to the importance of ethno botanical approach as useful measure for the discovery of new bioactive compounds (Boukef, 1986). However, further research is needed to identify the active agents responsible for the anticandidal, antidermatophytic, and antibacterial activities of *F. carica* latex.

The next work will be carried out on ethyl acetate fraction (LCMS, antiviral, cytotoxic and anti-inflammatory reactions).

## ACKNOWLEDGEMENTS

The authors are grateful to Mr. Mohamed Ben Salah, Laboratoire de Pharmacognosie Faculté de pharmacie Monastir and Mr. Mars Massoud, Laboratoire d'arboriculture, Ecole Supérieur d'Horticulture et d'Elevage, Chott Mariem Tunisia.

## REFERENCES

- Ahmad I and Beg AZ (2001). Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multidrug resistant human pathogens. *Journal of Ethnopharmacology*, **74**: 113-123.
- Bandelj D, Javornik B and Jakse J (2007). Development of microsatellite markers in the common fig, *Ficus carica* L. *Molecular Ecology Notes*, **7**: 1311-1314.
- Barry AL, Coyle MB, Thornsberry C, Gerland EH and Howkinson RW (1979). Methods of measuring zones of inhibition with the bauer-kirby disk susceptibility test. *Journal of Clinical Microbiology*, **10**: 885-889.
- Bassole IHN, Quattara AS, Nebie R, Quattra CAT, Kabore ZI and Traore SA (2003). Chemical composition and antibacterial activities of the essential

- oils of *Lippia chevalieri* and *Lippia multiflora* from Burkina Faso. *Phytochemistry*, **62**: 209-212.
- Bel Hadj Salah K, Ali Mahjoub M, Ammar S, Michel L, Millet-Clerc J, Chaumont JP, Mighri Z and Aouni M (2007). Antimicrobial and antioxidant activities of the methanolic extracts of three *Salvia* species from Tunisia. *Natural Product Research*, **20**: 1089-1097.
- Bel Hadj Salah K, Chraief I, Bannou F, Hammami M, Chemli R and Aouni M (2005). Variability in essential oil composition and antibacterial activity of Tunisian *Coridothymus capitatus*. *Journale de Microbiologie et Hygiène Alimentaire*, **17**: 50: 31-36.
- Boukef MK (1986). Les plantes dans la medecine traditionnelle Tunisienne. *Medecine traditionnelle et pharmacopée*, pp.203-204.
- Eloff JN (1998). Which extract should be used for the screening and isolation of anti microbial components from plants? *Journal of Ethnopharmacology*, **60**: 1-8.
- Giordani R, Trebaux J, Masi M and Regli P (2001). Enhanced antifungal activity of Ketoconazole by *Euphorbia characias* latex against *candida albicans*. *Journal of Ethnopharmacology*, **78**: 1-5.
- Karaman I, Sahin F, Gullus M, Ogutuc H, Sengul M and Adiguzel A (2003). Antimicrobial activity of aqueous and methanol extracts of *juniperus oxycedrus* L. *Journal of Ethnopharmacology*, **85**: 231-235.
- Kslev ME, Hartmann A and Bar-Yosef O (2006). Early domesticated fig in the Jordan Valley. *Science*, **312**: 1372-1374.
- Luilma AG, Sidrim JJC, Domingos TM, Cechinel Filho V and Rao VS (2005). *In vitro* antifungal activity of dragon's blood from *Croton urucurana* against dermatophytes. *Journal of Ethnopharmacology*, **97**: 409-412.
- Mars M (2003). Fig (*Ficus carica* L.) genetic resources and breeding. *Acta Hort.*, **605**: 19-27.
- Martins Teixeira D, Ferreira Patao R, Varela Coelho A and Teixeira da Costa C (2006). Comparison between sample disruption methods and solid-liquid extraction (SLE) to extract phenolic compounds from *Ficus*

- carica* leaves. *Journal of Chromatography A*, **1103**: 22-28.
- Mellou F, Lazari D, Skaltsa H, Tselepis AD, Kolisis FN and Stamatis H (2005). Biocatalytic preparation of acylated derivatives of flavonoid glycosides enhances their antioxidant and antimicrobial activity. *Journal of Biotechnology*, **116**: 295-304.
- Mitscher LA, Leu RP, Bathala MS, Wu WN, Beal JL and White R (1972). Antimicrobial agents from higher plants. I: Introduction, rational and methodology. *Lloydia*, **35**: 157-166.
- Omar I, Faraj Z, Adel K, Mansur F, Mohamed E, Wu Y, John FK and Yuanjiang P (2004). Microbial (1→3)-β-D-glucans from Libyan figs (*Ficus carica*). *Journal of Carbohydrate Polymers*, **58**: 181-184.
- Rio JL, Recio MC and Villar A (1988). Screening methods for natural products with antimicrobial activity: A review of the literature. *Journal of Ethnopharmacology*, **23**: 127-149.
- Sadder MT and Ateyyeh AF (2006). Molecular assessment of polymorphism local Jordanian genotypes of the common fig (*Ficus carica* L.). *Journal of Scientia Horticulturae*, **107**: 347-351.
- Sarang B, Anpurna K, Beenish K, Sheikh FA, Suri KA, Satti NK, Musarat A and Qazi, GN (2005). Immunosuppressive properties of an ethyl acetate fraction from *Euphorbia royleana*. *Journal of Ethnopharmacology*, **99**: 185-192.
- Shai R, Yoel K, Ruth R, Michael S and Raphael M (2001). Suppressors of Cancer Cell Proliferation from Fig (*Ficus carica*) Resin: Isolation and Structure Elucidation. *Journal of Natural Product*, **64**: 993-996.
- Singh G, Upadhyay RK, Narawaynan KP, Padmkumari and Rao GP (1993). Deuts Zeitschrift fur Pflanzenfrankeiten und *Pflanzenschutz*. **100**: 69-74.
- Tanaka Y (1992). The research for Bioactive compounds from micro-organisms. In: Omura, S. Springer Verlag, New York, pp.30-44.
- Vidaud J (1997). *Le figuier*. Edition centre technique interprofessionnelle des fruits et des légumes, Paris, p.264.
- Wang L, Jiang W, Ma K, Liang Z and Wang Y (2003). The production and research of fig (*Ficus carica* L.) in China. *Acta Horticulture*, **605**: 191-196.
- Zgoda JR and Porter JR (2001). A convenient microdilution method for screening natural products against bacteria and fungi. *Pharmaceutical Biology*, **39**(3): 221-225.
- Zouaoui, M (1992). Figue. In: La santé par les produits de la nature. ED. Zouaoui-Scandrani F., Tunis, pp.142-144.