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In vitro antimicrobial activity of aqueous and ethanolic extracts of leaves of *Ficus carica* collected from five different regions of Morocco

Galal Al Askari [1,3*], Azzeddine Kahouadji [1], Khadija Khedid [3], Laila Ouaffak [4], Mahjouba Mousaddak [2], Réda Charof [3], Zakaria Mennane [3]

1. *Laboratory of Botany, Mycology and Environment, FSR, Rabat, Morocco*
2. *Laboratory of Natural Substances and Extraction Organic, FSR, Rabat, Morocco*
3. *Laboratory of Bacteriology Medical, INH, Rabat, Morocco*
4. *Laboratory of Mycology Medical, INH, Rabat, Morocco*

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*Corresponding Author: Email: eco_galal@yahoo.com

Abstract
Aqueous and ethanolic extracts of leaves of *Ficus carica* collected from different regions in Morocco were screened for their antimicrobial activity against sixteen pathogenic bacterial strains and eight pathogenic yeast strains using agar well diffusion method and the determination of minimum inhibitory concentration was done by microtitration technique. The result obtained showed that the aqueous extract was active against Gram-positive bacteria more than Gram-negative bacteria but not active against yeast strains. Ethanolic extract had strong antibmicrobial activity, the maximum zone of inhibition was noted for Fez extract against *Staphylococcus epidermidis* (21mm) with MIC 25µg/ml and for Al Jadida extract against *Candida famata* (14.7mm) with MIC 50µg/ml. These results indicate to some benefits of fig leaves which can use to treatment the microbial infection.

Key words: Ficus carica- Leaves - Antimicrobial activity - Morocco

Introduction
Aromatic and medicinal plants are known sinceancient times, as antimicrobial against of plants. However, medicinal plants were not until the early 20th century that researchers are beginning presents the imported of them [1]. Several parties of plant used in traditional medicine are readily available in rural areas at relatively cheaper than modern medicine [2]. Many secondary metabolites are producer normally by Plants which constitute an important source of microbicides, pesticides and many pharmaceutical drugs. Plant products still remain the principal source of pharmaceutical agents used in traditional medicine [3,4].
Morocco has a long medical tradition and the traditional learning of plant remedies persisted until now. The art of healing is a part of the musulman tradition that reigned in this country [5]. Today, traditional medicines are a great part of modern health care systems in Morocco. Fig (*Ficus carica*) ones of these plants uses in medicinal traditional in morocco, ethnobotanical information on this plant was indicated by several works as Hseini [6], Said [7], Benchoabane [8], Lahssissene [9] and Tahraoui et al. [10].
The aim of this study was investigation the antimicrobial activity of Aqueous and Ethanolic extracts leaves of *Ficuscarica* collected from five different regions in morocco against a diverse range of organisms comprising Gram-positive and Gram-negative bacteria and yeasts.

Materials and methods

1. Plant material

The leaves of *Ficus carica* were collected in May –July 2010 from five Moroccan regions (Fez, Meknes, Al Jadida, Skhirat and Marrakech) and were identified by Dr. Azzeddine Kahouadji, Professor at the Faculty of Sciences of Rabat, laboratory of Botany, Mycology and environment. The leaves were shade dried at room temperature for fifteen days.

2. Preparation of plant extracts

2.1. Aqueous extract

The powdered of *Ficus carica* (50g) were extracted with boiling water (250 ml) for 30 min. After this step, the decoction was filtered and then freeze-dried (aqueous extract) [11].

2.2. Organic extracts

Ethanol extract was obtained by Soxhlet extraction of 100 g of aerial parts for 24 h in about 700 ml of solvent used. This extract, was concentrated to dryness and the residue was kept at 4° C [11] .

3. Microorganisms used

The test organisms used included: 16 bacteria strains(***Streptococcus pyogenes,Streptococcus sanguins, Staphylococcus epidermidis, Staphylococcus aureus*** Methicillin-resistant, ***Staphylococcus aureus, Pseudomonas aeruginosa,Acinetobacterbaumannii,Pseudomonas fluorescens,Salmonella enteritidis, Salmonella entericatyphimurium, Salmonella arizonae, Proteus mirabilis, Hafnia alveie resistant to*** β-lactamines, ***Yersinia enterocolitica, Escherichia .coli*** and ***Klebsiella pneumonia*** and 8 yeasts strains, ***Candida tropicalis*** (2), ***Candida famata***(2), ***Candida parapsilosis***(2) ***and Candida glabrata***(2) .These strains were collected from the National Institute of Health (NIH) Rabat - Morocco).

4. Culture media and Antimicrobial assay

Mueller-Hinton agar (MH) and Sabouraud Dextrose agar (SD) (Hi-Media, Bombay, India) were respectively used for bacteria and yeasts growth. Microbial cultures, freshly grown at 37°C/30°C were appropriately diluted in sterile normal saline solution to obtain the cell suspension at 10^5 CFU: ml.

To evaluate Antimicrobial activity, an agar well diffusion method was used as described by Nongpanga et al. [12], the organisms were spread on MH and SD agar plates by cotton swab. Wells of 6 mm diameter were punched into the agar medium and filled with 50 µl of plants extracts. The plates were incubated for 24 h at 37°C for bacteria and 48h at 30°C for yeast. Antimicrobial activity was evaluated by measuring the inhibition zone diameter against the test organism.

5. Minimum Inhibitory Concentration (MIC)

The determination of MIC of the plants extracts against microbial strains is performed according to the microtitration technique described by Eloff [13].

Results and discussion

The results of antimicrobial activity of the aqueous and ethanolic extracts of *Ficus carica* Leaves are indicated in table 1.It showed that the aqueous extracts of fig Leaves from different region have a better activity against Gram positive than Gram negative bacteria. Fez aqueous extract was the most active, it was active against 10 strains from 16 strains tested, and it was found to have maximum zone of inhibition against S***treptococcus sanguins***(14mm) while the minimum zone of inhibition was against ***Pseudomonas fluorescens***(8 mm).

The ethanolic extracts studied showed inhibition growth of most tested microorganisms with various degrees .The results obtained that the samples extract collected from Fez, was exhibited high antimicrobial activities against (14/16) strains tested and the inhibition diameter of this extract was between 8 – 21 mm, with an average of 14.5mm ; but this extract was only effective against ***Salmonella enteritidis*** and ***Klebsiella pneumonia,*** and there is no activity of all the extract tested against ***Salmonella*** spp2 and ***Hafnia alveie.*** Minimum inhibitory concentration results of ***Ficus carica*** leaves extracts are presented in Table 2, the aqueous extracts showed MIC between 50-100µg/ml. Most ethanolic extracts presented similar MIC against strains bacterial tested (50µg/ml), except the Fes extract who exhibited higher degree of anti-microbial activity (25µg/ml) against ***Staphylococcus*** as compared with the other extracts.

Table 1: Screening Antibacterial activity of *Ficus carica* leaves extracts collected from five different regions of Morocco

Bacterial strains tested	Inhibition zone diameters (mm)										
	Fez extract		Meknes extract		Al Jadida extract		Skhirat extract		Marrakech extract		Tetr-acycline 30µg/ml
	AE	EE	AE	EE	AE	EE	AE	EE	AE	EE	
SR	10	9.6	10	10.3	8	12	12	13.3	12	13.6	16
SS	14	17	8	13	9	14	12	8	8	8	18
SE	12	21	10	17	0	16	0	15	0	12.6	14
MSA	12	18	10	11	10	14	8	13	10	9.3	12
SA	10.6	14	10	13	12	13.6	8	15	0	13.6	13
PA	0	10.6	0	9	0	11	0	8	0	10.3	19
AB	10.6	15	0	12	0	12	0	19	0	13.3	16
PF	8	10	0	10	0	9	0	9.3	0	0	18
SLE	0	9.3	0	0	0	0	0	0	0	0	16
SLT	0	0	0	0	0	0	0	0	0	0	18
SLA	0	8	0	10	0	11	0	16	0	13	20
PM	11	12	10	12	0	11	0	12	0	10.3	16
HA	0	0	0	0	0	0	0	0	0	0	14
Y	12	14.6	10	10.7	8	10	0	12	0	10.6	13
EC	11	18.7	0	17	10	14.6	8	14	0	13.3	11
KP	0	8	0	0	0	0	0	0	0	0	17

SR:*Streptococcus pyogenes*, SS:*Streptococcus sanguins*, SE:*Staphylococcus epidermidis*, MSA, *Staphylococcus aureus* multiresistant, SA : *Staphylococcus aureus*, PA : *Pseudomonas aeruginosa*, AB:*Acinetobacterbaumannii*, PF : *Pseudomonas fluorescens*, SLE: *Salmonella enteritidis*, SLT: *Salmonella typhimurium*, SLA:*Salmonella arizonae*, PM: *Proteus mirabilis*, HA: *Hafnia alveie*, Y:*Yersinia enterocolitica*, EC: *Escherichia coli*, KP:*Klebsiella pneumonia*,AE: aqueous extracts, EE:ethanolic extracts,0: no activity

Table 2: Minimum inhibitory concentration of *Ficus carica*leaves extracts collected from five different regions of Morocco

Bacterial strains tested	MICs									
	Fez extract		Meknes extract		Al Jadida extract		Skhirat extract		Marrakech extract	
	AE	EE	AE	EE	AE	EE	AE	EE	AE	EE
SR	+	+	+	+ +	+	+ +	+	+ +	+	+ +
SS	++	+ +	+	+ +	+	+ +	+	+	+	+
SE	+	+ + +	+	+ +	-	+ +	-	+ +	-	+ +
MSA	+	+ + +	+	+	+	+ +	+	+ +	+	+
SA	+	+ +	+	+ +	+	+ +	+	+ +	-	+ +
PA	-	+ +	-	+	-	+ +	-	+ +	-	+ +
AB	+	+ +	-	+ +	-	+ +	-	+ +	-	+ +
PF	+	+ +	-	+ +	-	+	-	+ +	-	-
SL1	-	+	-	-	-	-	-	-	-	-
SL2	-	-	-	-	-	-	-	-	-	-
SLA	-	+	-	+ +	-	+ +	-	+ +	-	+ +
PM	+	+ +	+	+	-	+ +	-	+ +	-	+
HA	-	-	-	-	-	-	-	-	-	-
Y	+	+ +	+	+ +	+	+ +	-	+ +	-	+ +
EC	+	+ + +	-	+ +	+	+ +	+	+ +	-	+ +
KP	-	+	-	-	-	-	-		-	-

MIC: Minimum Inhibitory Concentration, +:100µg/ml, + +: 50µg/ml, + + +:25µg/ml,-: no activity

The results of antifungal activity leaves extracts of *Ficus carica* are presented in Table3. They showed no activity of aqueous extracts against the yeast strains tested. However, ethanolic extracts showed good inhibitory effects against most of yeast strains with inhibitions diameters zones situated between 8 and 14.5 mm, with an average of 7.5mm, the best activity was found with the extract of samples collected from Al jadida, it showed high activity against 6 strains. Minimum inhibitory concentration results of *Ficus carica* leaves extracts are presented in the same table 3. The ethanolic extracts presented similar MICs againstyeasts (*Candida famata1, Candida glabrata1*and *Candida glabrata2*) (50µg/ml), and showed MICs 100µg/ml against *Candida tropicalis*1and *Candida tropicalis*2.

Table 3: antifungal activity and minimum inhibitory concentration of *Ficus carica* leaves extracts collected from five different regions of Morocco

Yeasts strains tested	Aqueous extract	Inhibition zone diameters (mm) and MICs (µg/ml)										
		Fes extract		Meknes extract		Al Jadida extract		Skhirat extract		Marrakech extract		Streptomycin 12µg/ml
		EE	MIC	EE	MIC	EE	MIC	EE	MIC	EE	MIC	
CT1	0	8	+	8	+	8.7	+	8	+	8	+	18
CT2	0	8	+	8	+	8	+	8	+	8	+	22
CF1	0	13	++	12	+ +	14.5	+ +	14	+ +	13	++	14
CF2	0	0	-	0	-	0	-	0	-	0	-	17
CP1	0	8	+	0	-	8	+	0	-	0	-	16
CP2	0	0	-	0	-	0	-	0	-	0	-	10
CG1	0	12	+ +	12	+ +	12	+ +	11	+ +	11	+ +	12
CG2	0	11	+ +	10.6	+ +	10.6	+ +	8.7	+	10	+ +	15

CT1: *Candida tropicalis,* CT2: *Candida tropicalis,* CF1: *Candida famata,* CF2: *Candida famata,* CP1*: Candida parapsilosis,* CP2*: Candida parapsilosis,* CG1: *Candida glabrata,* CG2: *Candida glabrata,* 0: no activity.

The fig tree is one of the ancient plants used in the medicine; recently many studies have reported many of biological activity of extracts fig trees. The Gram- positive bacteria showed more sensibility for the plants extracts than Gram-negative bacteria [14].The week activity of the water extract against most bacterial strains investigated in this study is in agreement with previous works which show that aqueous extracts of plant generally showed little or no antibacterial activities [15-18].

Our results showed that ethanolic extract exhibited higher activity towards most the strains tested. The maximum activity was recorded against *Staphylococcus epidermidis, Staphylococcus aureus Meticilline Resistant* and *Escherichia coli,* the diameter of inhibition were respectively 21, 18 and 18.7mm, although MICs of three indicators was 25µg/ml. At this purpose Cutler and Wilson [19] have reported the activity of organic extract of fig leaves against *multiresistant Staphylococcus aureus*; Baby and Justin [20] and Mi-Ran et al. [21] showed a strong antibacterial activity of *Fiucs carica* extract leaves against oral bacteria.The results indicate the activity of fig extracts was more effective against Gram-positive than Gram- negative bacteria; this fact is in agreement with previous reports [22-26]. Nihal et al. [27], Alzoreky and Nakahara [28] have reported that the higher resistance of Gram-negative bacteria against plant extracts is credited to the presence of outer membrane lipopolysaccharides. Also these observations are likely to be the consequences of the differences in cell wall structure between Gram-positive and Gram-negative bacteria.Thus the Gram-negative outer membrane can acting as a barrier against many environmental substances, including antibiotics [29].

Many studies have reported the phytochemical analysis of fig leaves extracts, it's contains some phenolic compounds, which have pharmacological properties, namely flavonoids like rutin, quercetin, and luteolin, phenolic acids like ferrulic acid furanocoumarins like psoralen and bergapten, and also phytosterols like taraxasterol [30-32]. The antimicrobial activities of phenolic compounds of fig have been reported by some authors like: Lee and Jeong-Dan [33] and Mi-Ran et al [21].The results obtained indicate the therapeutic virtue of fig leaves as an antimicrobial agents against some microbial infection, such infection by methicillin-resistant *Staphylococcus aureus* which recognized as a global nosocomial problem [34, 35].

The aqueous extract showed no activity against yeasts strains tested, but ethanolic extracts were active against 75% of strain tested, however, the most of activity was weak (diameter of inhibition between 7 mm and 10 mm) and the maximum inhibition zone was fund with Al Jadida extract against *Candida famata*(14.7mm with MIC 50 µg/ml), the antifungal activity of plants extract always indicate the weak or moderate activity [36-40]. The lack of antifungal activity could be explain by the parts of the plants, used method of extraction, the type of solvent, and possibly the time of collection [41].

Conclusion

Considering that results obtained in this work, we can be concluded that the leaves of *Ficus carica* collected from five different regions of Morocco have shown strong antimicrobial activity, where the extract of samples collected from Fez has a higher activity. The aqueous extracts of fig leaves have a Good activity against Gram positive than Gram negative bacteria. However there's no activity of aqueous extracts against the yeast strains tested. The ethanolic extracts presented high antimicrobial properties against Gram positive and Gram negative, the highest MIC value was recorded with the extract of samples of fez. Also, the ethanolic extracts were active against the most of yeast strain. The results indicated the importance of fig leaves extracts as antibacterial agent especially the action of these extracts were bactericidal against the bacteria and yeast tested, these finding have been encouraging to several applications as treatment of bacterial infections and food preservation. For that, suggest further investigations on these extracts like chemical compositions and conservation.

References

1. Mahesh, B., Satish, S., *World J. Agri. Sci.* 4 (2008) 839.
2. Mann, A., Banso, A., Clifford, LC., *Tanzania J. Health Res.* 10 (2008) 34.
3. Ibrahim, MB., *J. Pharma. Devpt* 2 (1997) 20.
4. Ogundipe, O., Akinbiyi, O., Moody, JO., *Nigeria J. Natural Products & Medicine* 2 (1998) 46.
5. Bellakhdar, J., Ed. Ibis Press, Paris(1997) 11.
6. Hseini, S., Thèse du doctorat(2008).
7. Said, MK., Thèse du doctorat (1995).
8. Benchoabane, A., Thèse du doctorat (1995).
9. Lahssissene, H., Thèse du doctorat (2010.
10. Tahraoui, A., El-Hilaly, J., Israili, ZH., Lyoussi, B., *J. Ethnopharmacol* 110 (2007)105.
11. Harbone, J.B., Halsted press, John Wiley and Sonc, Inc, New York (1976) 1.
12. Nongpanga, K., Aporn, W., Duangtip, M. Sukon ,T., *Kmitl Sci. Tech.* J8 (2008) 1.
13. Eloff, JN., Plant Medical, 64 (1998) 711.
14. Selvi, M., Selvaraj, R.,Anandhi, C., *Utilin Seaweed Res*23 (2001)59.
15. Koduru, S., Grierson, DS., Afolayan, A. *J., Pharamacol.* Biol. 44(2006). 284.
16. Aliero, A.A., Grierson, DS., Afolayan, A *J., Res. J. Bot.* 1 (2006)129.
17. Ashafa, A, Grierson, D., Afolayan, A., *J. Biol. Sci* 8 (2008)1062.
18. Aiyegoro, O.,Akinpelu, D., Afolayan, A., Okoh, A., *J. Biol. Sci.* 8 (2008) 356.
19. Cutler, R.,Wilson, P., *Bri. J. Bio. Sci. 61* (2004)*5.*
20. Baby, J., Justin, S., Inter. *J. Pha. Tech Res.* 3 (2011) 08.
21. Mi-Ran, J., Hye-Young, K., Jeong-Dan, Cha, *J. Bact. Viro.* 39 (2009) 97.
22. Vlientinck, AJ., VanHL., Totte, J., Lasure, A., Vanden, BD., Rwangabo PC., Mvukiyumwami J., *J. Ethnopharmacol.,* 46 (1995) 31.
23. Lin, J., Opoku, AR., Geheeb-Keller, M., HutchingsAD., Terblanche, SE.,Jager, A.,Van, J., *J. Ethnopharmacol* 68 (1999) 267.
24. Didem, DO., Nilufer, O., Berrin, O.,Fatma, E., *Turk. J. Biol* 33 (2009). 341.
25. Oskay, M.,Sar, D., Pharm. Biol., 45 (2007) 176.
26. Rabe, T.,Van Staden, J., *J. Ethnopharmacol* 56 (1997) 81.
27. Nihal, T., Sedat, V., Ferda, S. Gokce, P, *Molecules*,12 (2007) 484.
28. Alzoreky, N., Nakahara, K., *J. Food Microbial,* 80 (2003) 223.

29. Tortora, G., Funke B.,Case, C., "Microbiology: an Introduction" Benjamin Cummings; San Francisco (2001) 88.
30. Vaya, J., Mahmood, S., *Biofactors* 28 (2006)169.
31. Ross, J., Kasum, C.,*Annu. Rev. Nutr.* 22 (2002) 19.
32. Teixeira, D., Patão, R., Coelho A., Costa C., *J. Chromatogr.* 1103 (2006) 22.
33. Lee, Y., Jeong-Dan C., Kor. *J. Microbiol. Biotechnol.* 38 (2010) 405.
34. Jorgensen, M., Givney R., Pegler, M., Vickery, A., Funnell G., *J. Clin. Microbiol.* 34 (1996) 398.
35. O'Connell, N., Humphreys, H., Pidgeon, C., Smyth, E., *Clin. Microbiol. Infect.* 9 (2003) 578.
36. Taniguchi, M., Kubo, I., *J. Natural Products*, 56 (1993)1539.
37. Meena, M., Sethi V., *J. Food Science and Technol31* (1994) 68.
38. Saeed, A., Ibrahim, N., Bashandy, S. El-gengaihi, S., *Bul. Fac. Pharm., Cairo Uni.33* (1995). *105.*
39. Ela, M., El-shaer, N., Ghanem, NB., *Pharmazie*, 51 (1996) 993.
40. Alasbahi, R., Safiyeva, S., Craker, L., *J. Her. Sp. Med.* P. 6 (1996)75.
41. Omar, J., Hamzaa, C., Van den, B., Mecky, I., Matee, M., Moshi F., Mik, M., *J. Ethnopharm.* 108 (2006) 124.