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Polyphenols content and antimicrobial potential of extracts from leaves of *Celtis australis*

Soukaina El Maliki, Najoie Filali-Ansari, Said El Khyari, Ahmed El Abbouyi*

Laboratory of Biochemistry, Nutrition and Valorization of Natural Resources.
Faculty of Sciences. PO 20. El Jadida 24000. Morocco.

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Abstract: The dichloromethane, ethyl acetate, butanol and methanol extracts from leaves of *Celtis australis*, used to treat different disorders in Moroccan traditional medicine, were screened for their contents of total polyphenols, flavonoïds and condensed tannins and antimicrobial activity against seven bacterial strains (*Staphylococcus aureus*, *Bacillus cereus*, *Bacillus sp*, *Listeria ivanovii*, *Escherichia coli*, *Citrobacter freundii* and *Salmonella sp*) and three fungi (*Candida albicans*, *Candida tropicalis* and *Aspergillus niger)*, using Ampicillin and Fluconazole as references. The antimicrobial activities of plant extracts were assessed by the disc diffusion and minimum inhibitory concentration (MIC) methods. All values are expressed as mean ± SD. The obtained results showed that the butanol extract exhibited significant and dose-dependent antibacterial activity against *S. aureus*, *B. cereus*, *Bacillus sp* and *Salmonella sp*. The other extracts exerted moderate antibacterial activity, except methanol extract that inhibited significantly *Listeria ivanovii*. In the same conditions, the butanol, dichloromethane and ethyl acetate extracts showed significant and dose-dependent antifungal activity against all tested fungi, although at different extents, while methanol extract exhibits moderate effect. The plant extracts yielded important polyphenolic contents assessed by total polyphenols, flavonoïds and condensed tannins. A positive linear correlation was established between these compounds and the antimicrobial activity of plant extracts. These results are corroborating the traditional medicine use of *Celtis australis* which could be a good candidate for further studies.

Key words: Antibacterial, Antifungal, Leaves, *Celtis australis*, Polyphenols

INTRODUCTION

Drug resistance to human pathogenic microorganisms, due to indiscriminate use of antibiotics, is increasingly reported in developing as well as developed countries[1]. This public health problem complicated seriously the treatment of infectious diseases usually and in immune-compromised, AIDS and cancer patient's particularly[2].

Thus, the emergence of multiple drug resistance of pathogenic organisms necessitated a search for new antimicrobial substances from natural sources including plants. Nature has been a source of medicinal plants for thousands of years and since the beginning of man. All over the word, traditional medicine systems used for a long time plants to threat various human and animal illnesses and disorders including infectious diseases. So far, more than 100,000 biologically active secondary plant metabolites have been isolated from higher plants[3]. Therefore, traditional knowledge of medicinal plants and their use by population are not only useful for conservation of traditional knowledge and biodiversity but also for primary health care and drug development. In Morocco, the use of traditional medicine for the treatment of various disorders and illnesses has been practiced for generations and a large part of the population in the country uses medicinal plants for its day to day healthcare needs[4]. For this reason, such plants should be investigated to better understand their properties, safety and efficiency.

Cannabaceae is a large family, containing about 15 genera and 200 species. The largest genus, *Celtis*, includes about 60 species. Among these species is *Celtis australis*, commonly known as the European nettle tree, Mediterranean hackberry or honeyberry, a deciduous tree endemic to southern Europe, North Africa, and south-western Asia[5,6].The leaves and fruits are astringent, lenitive and stomachic. Decoction of both leaves and fruits is used in the treatment of amenorrhea, heavy menstrual and inter-menstrual bleeding and colic. The decoction can also be used to astringe the mucous membranes in the treatment of diarrhea, dysentery and peptic ulcers. The paste obtained from bark is used as remedy for bone fracture and also applied on pimples, contusions, sprains and joint pains[4,7,8].

The objective of the present study was to determine the phenolic profile and to evaluate the antimicrobial potential of the dichloromethane, ethyl acetate, butanol and methanol extracts obtained from leaves of *C. australis*.

MATERIAL AND METHODS

Plant material: *C. australis* was collected during spring 2017 in El Jadida city (Morocco). Plant material was authenticated by specialist. A voucher specimen (reference CA1/13) is kept on file in our laboratory

Preparation of plant extracts: The air-dried leaves (200g) of *C. australis* were powdered mechanically and sieved using a fine muslin cloth. The obtained powder was then exhaustively extracted three times with methanol, by maceration in a hermetically closed glass vessel for 24 h at room temperature (25°C) under occasional shaking. After filtration, the resultant extract was concentrated under reduced pressure at 40°C.The methanol crude extract (28.6g) was solubilized in distilled water and extracted successively with equal volumes of three organic solvents of increasing polarity. Each extract was evaporated to dryness under vacuum giving three fractions: dichloromethane (2g), ethyl acetate (1.4g) and butanol (9.2g). All extracts were stored at +4°C until use.

Chemicals: Butylated hydroxytoluene (BHT), catechin, gallic acid, quercetin, vanillin, 1,1-diphenyl-2-picrylhydrazyl (DPPH), Folin-Ciocalteus's phenol reagent, sodium carbonate, sodium nitrite, linoleic acid, cupric sulphate, EDTA (ethylne diamine tetracetic), Tween 20, trichloracetic acid (TCA), thiobarbituric acid, n-butanol and other chemicals used were of analytical grade and were obtained from either Sigma-Aldrich or Merck.

Phenolic profile

Total polyphenols content: Quantification of total polyphenols was made using a modified method as previously described[9].

Total flavonoïds content: The flavonoïds content of extracts was quantified using the modified method as previously mentioned[9].

Total tannins content: The condensed tannins content was performed according to the modified method as previously reported[9].

Antimicrobial screening

Microorganisms: The antibacterial screening was conducted against four Gram positive bacteria: *Staphylococcus aureus* (ATCC 25923), *Bacillus sp* (CIP 104717), *Bacillus cereus* (ATCC 33019) and *Listeria ivanovii* (ATCC 19119), and three Gram negative bacteria: *Escherichia coli* (CIP 54127), *Citrobacter freundii* (ATCC 8090) and *Salmonella sp*.

For antifungal test, two yeasts (*Candida albicans* and *Candida tropicalis*) and one filamentous fungus (*Aspergillus niger*) were used.

All the microorganisms were procured from Pasteur Institute (Casablanca, Morocco). They were maintained by subculturing periodically and preserved at +4°C prior to use. For inocula preparations, bacteria and fungi were incubated for 24 h in Mueller Hinton Agar medium (MHA) and 3 days in Potato Dextrose Agar medium (PDA) respectively.

Antimicrobial test: Susceptibility of the microorganisms to plant extracts was determined by employing the disc-diffusion method. The bacterial and yeasts cultures in the exponential phase of growth or fungal spore solution were spread on MHA or PDA plates in order to give a population of approximately 10^8 CFU/plate.

Commercial paper discs (6mm in diameter), sterilized at 120°C for 15min, were first impregnated separately with 20µl of three concentrations of each extract (80, 200 and 400mg/ml) and were then deposed on the surface of the plates. The plates were kept at +4°C for 2h and then incubated for 24h (bacteria and yeasts) or 3 days (filamentous fungus) at 37°C under aerobic conditions and the diameter of the zone of inhibition around each disc was then measured and recorded. Negative control was set up with equivalent quantities of solvents used in the extraction process. Ampicillin (10µg/disc) and Fluconazole (10µg/disc) were used as standards. All the experiments were performed in triplicate and the results (mm of the zone of inhibition) were expressed as mean value ± standard deviation.

Antimicrobial efficiency of extracts was evaluated according to the following scale:

Ø ≤ 8mm : No significant antimicrobial activity

8 < Ø ≤ 12mm : Moderate antimicrobial activity

12< Ø ≤ 14mm : Significant antimicrobial activity

Ø >14mm : Very significant antimicrobial activity

Determination of Minimum Inhibitory Concentration (MIC): The MIC was determined by adopting the tube dilution method. One ml of the bacterial suspension (1.5×10^6 CFU/ml) was inoculated into each tube. Control tubes were inoculated with the same volume of 80% methanol in sterile distilled water. All tubes were incubated at 28°C for 24 h. The lowest concentration that did not allow any detectable bacterial growth when compared with the control was considered as the minimum inhibitory concentration (MIC).

Statistical analysis: The results are expressed as Mean ± SD. The data obtained from various groups were statistically analyzed using one-way ANOVA followed by Tukey's Multiple Range Test. Values with $p<0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

The increase in incidence of new and re-emerging infectious diseases requires the search of new antimicrobial compounds with divers and novel mechanism of action. Plants produce many substances for self-defense against parasites and microorganisms. Therefore, plants could an endless of new antimicrobial agents.

There are about 47,000 plants species in Morocco out which 7,500 plants species are medicinal value. However, only 800 plants species are used in preparation of herbal drugs[10,11]. Besides, the originality of the Moroccan flora is illustrated by its high percentage of endemism (20%)[12]. This phyto-diversity allows Moroccan population to have a long traditional knowledge on herbal medicine use[4,13].

The present study was designed to evaluate the polyphenols content and the antimicrobial potential of four different extracts obtained from leaves of *C. australis*.

Extraction process: The yields of the extracts from *C. australis* leaves are presented in **Table 1**. The maximum yield was obtained with the methanol extract (14.3%) followed by butanol extract (4.6%), ethyl acetate extract (1.0%) and dichloromethane extract (0.7%).

Table 1: Percentage yields of extracts from leaves of *Celtis australis*

Solvent	Yield (w/w) (%)
Methanol	14.3
Butanol	4.6
Dichloromethane	1.0
Ethyl acetate	0.7

Phytochemical profile: The contents of total polyphenols, flavonoïds and condensed tannins of extracts from leaves of *C. australis* are reported in **Table 2**. The results revealed high level of these compounds that are distributed mainly in polar solvent extracts. The ethyl acetate extract exhibited highest amount of total polyphenols (193mg GAE/g of dry extract) and flavonoïds (87mg CE/g of dry extract) that represent 45% of total polyphenols. The methanol extract contains 172.5mg GAE/g of dry extract of which flavonoïds (51mg CE/g of dry extract) represent 30%. The amounts of total polyphenols in the butanol extract reach 143.3mg GAE/g of dry extract of which flavonoïds (65mg CE/g of dry extract) represent 45%. Compared to the previous extracts, the total polyphenols content in the dichloromethane extract is relatively low (21.2mg AGE/g of dry extract) of which flavonoïds

(7.8mg CE/g of dry extract) represent 37%. Similarly, condensed tannins are mainly found in polar extracts. The methanol extract exhibited highest amount of condensed tannins (516.8 mg TAE/g of dry extract), followed by the butanol extract (273.7 mg TAE/g of dry extract) and the ethyl acetate extract (223.3mg TAE/g dry extract) whereas the dichloromethane extract contains low amount of condensed tannins (4.4mg TAE/g of dry extract).

Table 2: Polyphenolic contents of extracts from leaves of *C. australis*

	Ethyl acetate extract	Methanol extract	Butanol extract	Dichloromethane extract
Total polyphenols[a]	193	172.5	143.3	21.2
Flavonoïds[b]	87	51	65	7.8
Condensed tannins[c]	223.3	516.8	273.7	4.4

[a]mg Gallic acid equivalent/g of dry extract;[b]mgCatechin equivalent/g of dry extract
[c]mg Tannic acid equivalent/g of dry extract

Although different from our previous work[14], these results validate the plenty of polyphenolic compounds in this plant. It is well known that the content of secondary metabolites varies according to the season and the growth phase of the plant. This variation was reported by a study that shows a fluctuation with time of phenolics content in *C. australis* leaves. Young leaves of *C. australis* were found to contain the highest amounts of phenolics that rapidly decreased until mid-May and after this date the level of phenolics fluctuated but showed no discernible trend. This general trend of high amounts of phenolics in the early growing season and a fast decline affected both caffeic acid derivatives and flavonoïds[15].

Antibacterial potential: The antibacterial activity of the plant extracts, assessed by diameter of inhibition, is reported in **Table 3.** The most antibacterial effect was exerted by the butanol extract followed by the ethyl acetate and methanol extracts while the dichloromethane extract exhibited no-significant effect. The butanol extract showed a broad spectrum antibacterial activity in a dose-dependent manner. At low concentrations (1.6 and 4mg/disc), a moderate antibacterial effect ($8<Ø\leq12$mm) is obtained against all bacteria tested except *E. coli* and *B. sp*. At high concentration (8mg/disc), a significant antibacterial effect ($12<Ø\leq14$mm) was observed against *S. sp*, *S. aureus*, *B. sp* and *B. cereus*, whereas *E. coli*, *C. freundii*, and *L. ivanovii* were moderately inhibited. In the same conditions, the methanol extract inhibited moderately, in a dose-dependent manner, all bacteria tested except *L. ivanovii* that was significantly inhibited at 8mg/disc. Similar results were obtained with the ethyl acetate extract that exhibited a dose-dependent but moderate antibacterial effect against all bacteria tested. The dichloromethane inhibited moderately all bacteria tested only at 8mg/disc. It is to note, that Ampicillin, used as standard at 10µg/disc, inhibited very significantly all bacteria strains tested, except *Salmonella sp* that was moderately inhibited while negative control (solvents) exhibited no significant effect (data not shown).

Table 3: Antibacterial effect of leaves extracts from *Celtis australis*

	Diameter of inhibition (mm)												
	Dichloromethane extract (mg/ml)			Ethyl acetate extract (mg/ml)			Butanol extract (mg/ml)			Methanol extract (mg/ml)			Ampicillin (µg/ml)
	1.6	4	8	1.6	4	8	1.6	4	8	1.6	4	8	10
Escherichia coli	5.0±0.5	7.5±0.5	8.5±0.5	9.0±0.5	8.5±0.5	9.5±0.5	6.0±0.5	9.5±0.5	10.5±0.5	5.0±0.5	9.5±0.5	10.5±0.5	21.5 ± 1.5
Salmonella sp	6.5±0.5	7.5±0.5	8.5±0.5	7.5±0.5	8.5±0.5	9.5±0.5	9.5±0.5	9.5±0.5	12.5±0.5	6.5±0.5	8.5±0.5	11.5±0.5	11.5 ± 0.5
Citrobacter freundii	6.5±0.5	8.0±1.0	9.5±0.5	8.5±0.5	8.5±0.5	10.5±0.5	9.5±0.5	10.5±0.5	11.5±0.5	9.5±0.5	9.5±1.5	11.0±1.0	21.0 ± 1.0
Staphylococcus aureus	5.5±0.5	8.0±1.0	9.5±0.5	7.5±0.5	9.5±0.5	12.0±1.0	9.5±0.5	10.5±0.5	13.5±0.5	6.5±0.5	9.0±1.0	9.5 ± 0.5	28.5 ± 0.5
Bacillus sp	6.5±0.5	8.0±1.0	9.5±0.5	8.5±0.5	9.5±0.5	10.5±0.5	6.5±0.5	10.5±0.5	13.0±1.0	8.5±0.5	9.5±0.5	10.0±1.0	33.5 ± 0.5
Listeria ivanovii	6.5±0.5	8.5±0.5	9.5±0.5	9.5±0.5	10.5±0.5	12.0±1.0	9.5±0.5	10.5±0.5	11.5±0.5	9.5±0.5	10.5±0.5	12.5±0.5	21.0 ± 1.0
Bacillus cereus	5.5±0.5	6.5±0.5	9.0±0.5	8.0±1.0	9.5 ± 0.5	10.5±0.5	8.5±0.5	10.5±0..5	13.5±0.5	7.5±0.5	8.5 ± 0.5	9.5 ± 0.5	14.5 ± 0.5

The extent of antibacterial activity seems to depend on the nature of the solvent of extraction. Significant effects were observed with the polar solvents (butanol, ethyl acetate and methanol), while the effect produced by non-polar solvent (dichloromethane) is no-significant. This difference of distribution is probably correlated with the degree of solubility of the active compounds according to the polarity of the solvent of extraction. It can be concluded that the major part of the compounds having antibacterial activity is essentially distributed in the butanol extract, moderately in the ethyl acetate and methanol extracts and weakly in the dichloromethane extract. This supposition is consistent with MIC values (**Table 4**) showing that the butanol extract is the most effective (5mg/ml) against all tested bacterial strains followed by the ethyl acetate extract (5 to 10mg/ml), the methanol extract (10 to 20mg/ml) and the dichloromethane extract (10 to 40mg/ml). It should be noted that Ampicillin, used as a reference, inhibited all bacteria tested with MICs ranging from 1 to 2µg/ml.

These results are in agreement with our previous study which has reported the antibacterial effect of hydro-methanolic extracts of leaves and seeds of *C. australis*[14], as far as various extracts from this plant against *S. aureus, P. aeruginosa, E. coli* and *B. subtilis*[16], *S. aureus* and *P. aeruginosa*[8], *P. syringe*[17]and *S. aureus, L. monocytogenes, P. aeruginosa* and *E. coli*[18].

Table 4: Antibacterial MICs of extracts from *C. australis*

Strains	Methanol mg/ml	Butanol mg/ml	Ethyl acetate mg/ml	Dichloromethane mg/ml	Ampicillin µg/ml
Escherichia coli	10	5	5	40	12
Bacillus sp	10	5	5	10	8
Citrobacter freundii	20	5	10	10	4
Staphylococcus aureus	20	5	10	40	8
Listeria ivanovii	20	5	10	10	4
Bacillus cereus	20	5	10	10	12
Salmonella sp	20	5	10	10	1

Antifungal potential: As shown in **Table 5,** a significant and dose-dependent antifungal effect was obtained with all extracts tested. At high concentration (8mg/disc), the most antifungal activity was obtained with the butanol extract that inhibited very significantly *A. niger* (18.5mm) and *C. albicans* (15.5mm) and significantly *C. tropicalis* (12.5mm). In the same conditions, the dichloromethane and ethyl acetate extracts exhibited a very significant effect against *C. tropicalis* (19.5mm and 15.5 mm respectively) and significant effect against *A. niger* (12.5mm each). Fluconazole, used as standard at 10µg/disc, inhibited very significantly *C. albicans (20mm)* and *C. tropicalis* (17.5mm) and moderately *A. niger* (11.5mm).

The MIC values of extracts, reported in **Table 6**, showed that all extracts tested exhibited a very significant effect against *C. tropicalis* (0.625 to 1.25µg/ml) and moderate effect against *C. albicans* and *A. niger* (5 to 10mg/ml) except the dichloromethane extract that inhibited *C. albicans* very significantly (1.25mg/ml). Fluconazole, used as antifungal standard, inhibited all tested fungi with MIC varying from 4 to 12µg/ml.

Table 5: Antifungal effect of leaves extracts from *Celtis australis*

	Diameter of inhibition (mm)												
	Dichloromethane extract mg/disc			Ethyl acetate extract mg/disc			Butanol extract mg/disc			Methanol extract mg/disc			Fluconazole µg/disc
Strains	1.6	4	8	1.6	4	8	1.6	4	8	1.6	4	8	10
Candida tropicalis	9.5 ± 0.5	13.5 ± 0.5	19.5±0.5	5.5 ± 0.5	11.5±0.5	15.5± 0.5	9.5 ± 0.5	10.5 ± 0.5	12.5 ± 0.5	9.5 ± 0.5	11.5 ± 0.5	11.5±0.5	17.5 ± 2.5
Aspergillus niger	7.5 ± 0.5	11.5 ± 0.5	12.5±0.5	7.5 ± 0.5	9.5 ± 0.5	12.5±0.5	11.5±0.5	14.5 ± 0.5	18.5 ± 0.5	5.0±0.5	5.0±0.5	9.5 ± 0.5	11.5 ± 1.5
Candida albicans	5.0 ± 0.5	5.0 ± 0.5	9.5 ± 0.5	6.5 ± 0.5	7.5 ± 0.5	9.5 ± 0.5	10.5±0.5	12.5 ± 0.5	15.5 ± 0.5	5.0 ± 0.5	5.0 ± 0.5	5.0 ± 0.5	20.0 ± 1.0

Table 6: Antifungal MICs of extracts from *Celtis australis*

Strains	Methanol mg/ml	Butanol mg/ml	Ethyl acetate mg/ml	Dichloromethane mg/ml	Fluconazole µg/ml
Candida tropicalis	0.625	0.625	1.25	0.625	4
Candida albicans	10	5	10	1.25	8
Aspergillus niger	5		5	10	12

These results are corroborated by previous studies demonstrating the antifungal activity of various solvent extracts from different parts of *C. australis* against *C. albicans, C. tropicalis* and *A. niger*[14] and against *C. albicans, C. parapsilosis*, and *R. mucilaginosa*[18]. This potential was also shown with leaves extract of *C. africana* against *Cryptococcus neoformans*[19].

The antimicrobial effect revealed in this study can be related to the presence of polyphenolic compounds, the secondary plant metabolites that are ubiquitously present in plants and plant products[20]. Several publications have reported that plant polyphenols exhibited various activities among which antimicrobial effect[21,22]. These compounds contribute to the overall antimicrobial activity of plants mainly due to their broad spectrum by suppressing microbial virulence factors such as inhibition of biofilm formation, reduction of receptor ligands adhesion and bacterial toxins neutralization[23], by inducing cell wall and cytoplasmic membrane perturbations[24] and/or by interference with certain microbial metabolic processes, modulation of signal transduction or gene expression pathways[25].

CONCLUSION

In the present study, the antimicrobial effect of four different extracts of leaves from *C. australis* was evaluated by the measurement of inhibition diameters and the quantification of MICs. Based on the results, it could be conclude that all tested extracts showed a relative antimicrobial effect which can be related to the presence of polyphenol compounds. Our results were consistent with traditional uses of *C australis* which is prescribed against gastro-intestinal ailments. Furthermore, the detection of antimicrobial activities indicates that this plant may be a source for antibacterial and antifungal drugs. Bioassays-guided research are in progress to reveal new, renewable and more potent compounds from this plant.

REFERENCES

1. R.J. Fair, Y. Tor. Antibiotics and bacterial resistance in the 21st Century. Perspect Medicin Chem. 2014; 6: 25–64.
2. C.L. Ventola. The antibiotic resistance crisis. Part 1: Causes and threats. Pharm Therap. 2015; 40(4): 277–283.
3. M. Mazid, T.A. Khan, F. Mohammad. Role of secondary metabolites in defense mechanisms of plants. Biol Med. 2011; Special Issue 3(2): 232-249.
4. J. Bellakhdar. La pharmacopée traditionnelle marocaine (Moroccan traditional pharmacopeia). Ibis Press ed, Paris (France).1997. 519-520
5. T.G. Tutin. Celtis. In Flora Europaea. Volume 1. Cambridge University Press ed, UK, 1972: 65–66.
6. U. Quattrocchi. CRC World dictionary of plant names. Volume 4. CRC Press ed, Florida (USA). 2000.
7. R.D. Gaur. Flora of district Garhwal North West Himalaya. Trans Media House ed. Srinagar, Garhwal, India, 1999. 84-85.
8. A. Showkat, S. Rajendra, M. Surabhi, G. Ankur. Antibacterial activity of *Celtis australis* by in vitro study. Int J Pharma Pharmaceut Sci. 2012; 4:629-31.
9. N. Filali-Ansari, A. El Abbouyi, S. El Khyari. Antioxidant properties of leaves and seeds hydromethanolic extracts from *Celtis australis*. J Chem Biol Phys Sci. Sec. B. 2015. 5(3): 2834-2843.

10. M. Fennane, M. Ibn Tattou, J. Marthez, A. Ouyahya, J. El Oualidi. Flore pratique du Maroc. Manuel d'identification des plantes vasculaires. Volume 1. Institut Scientifique; Série botanique n° 36. Rabat (Maroc).1999.
11. M. Fennane, M. Ibn Tattou. Statistiques et commentaires sur l'inventaire actuel des plantes vasculaires du Maroc. Bulletin de l'Institut Scientifique, Section sciences de la vie. 2012; 34:1-9.
12. H. Rankou, A. Culham, S.L. Jury, M.J.M. Christenhusz. The endemic flora of Morocco. Phytotaxa. 2013; 78:1-69.
13. A. Sijelmassi. Plantes médicinales du Maroc. Edited by Le Fennec. Rabat (Maroc).1991.
14. N. Filali-Ansari, A. El Abbouyi, S. El Khyari, R. Eddoha. Antibacterial and antifungal activities of seeds and leaves extracts from *Celtis australis*. J Chem Biol Phys Sci. Section B. 2015; 5(2):1401-1407.
15. V. Sommavilla, D. Haidacher-Gasser, M. Sgarbossa, C. Zidorn. Seasonal variation in phenolics in leaves of *Celtis australis* (Cannabaceae). Biochem System Ecology. 2012; 41: 110–114.
16. R. Badoni, D.K. Semwal, U. Rawat. Fatty acid composition and antimicrobial activity of *Celtis australis* L. fruits. J SciRes. 2010; 2 (2):397-402.
17. B. Elkhalfi, A. Essari, A. Serrano, A. Soukri. Antibacterial activity of plant methanolic extracts on a field isolate of *Pseudomonas syringae* pv tomato from the Casablanca region (Morocco). Adv Biosc Biotechnol. 2013; 4: 1-9.
18. A. Ota, A. MiklavcicVišnjevec, R. Vidrih, Z. Prgomet, M. Necemer, J. Hribar, N. GundeCimerman, S. SmoleMožina, M. Bucar-Miklavcic, N. PoklarUlrih. Nutritional, antioxidative, and antimicrobial analysis of the Mediterranean hackberry (*Celtis australis* L.). Food Sci Nutr. 2017; 5(1): 160–170
19. T.A. Mokoka, L.J. McGaw, J.N. Eloff. Antifungal efficacy of ten selected South African plant species against *Cryptococcus neoformans*. Pharm Biol. 2010; 48(4):397-404.
20. R. Ross Watson. Polyphenols in plants. Isolation, purification and extract preparation. Academic press Elsevier ed. New York (USA). 2014
21. M. Daglia. Polyphenols as antimicrobial agents. Curr Opin Biotechnol. 2012; 23(2):174-81.
22. E. Coppo, A. Marchese. Antibacterial activity of polyphenols. Curr Pharm Biotechnol. 2014; 15(4):380-90.
23. A.J. dos Reis Albuquerque, P.M. de Freitas e Silva, A.L. Furtado de Almeria Cavalcante, F. Correia Sampaio. Polyphenols as a source of antimicrobial agents against human pathogens. In plant extracts: Role in agriculture, health effects and medical applications. Edited by A. Giordano and A Costs. Nova Science Publishers. 2013
24. S. Pai-Wei, Y. Cheng-Hong, Y. Jyh-Ferng, S. Pei-Yu, C. Li-Yeh. Antibacterial activities and antibacterial mechanism of *Polygonum cuspidatum* extracts against nosocomial drug-resistant pathogens. Molecules. 2015; 20, 11119-11130

25. C. Omojate Godstime, O. Enwa Felix, O. Jewo Augustina, O. Eze Christopher. Mechanisms of antimicrobial actions of phytochemicals against enteric pathogens: A review. J. Pharmac Chem Biol Sc. 2014; 2(2):77-85.

Corresponding author: Ahmed El Abbouyi*

Laboratory of Biochemistry, Nutrition and Valorization of Natural Resources.

Faculty of Sciences. PO 20. El Jadida 24000. Morocco.

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