ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Antibacterial Activity of Essential Oils of *Chamaerops humilis* (Arecaceae) on Some Pathogenic Bacteria

HASNAOUI Okkacha*[1, 2], ADLI Djallal Edine[1] and SENNOUR Raja[1]

[1] Department of Biology, Faculty of Sciences and Technology, University of Saïda 20000, Algeria
[2]Ecological and natural ecosystem Managements Laboratory University of Tlemcen - 13000, Algeria

ABSTRACT

The Arecaceae family is represented in the west Mediterannean a monospecific genus: *Chamaerops humilis* L. This species is native to the Mediterranean basin. It is used by people in many countries for its medicinal virtues. To contribute to a better appreciation of this taxon was tested in vitro the inhibition of the essential oils in the growth of certain pathogenic bacteria. The extraction of essential oils (E.O) by different processes provided mixed returns. They range from 5% to 2.7% in the leaves and 1.5% to 0.4 % in the fruit by extraction using soxlhet; 1.5% for the leaves to 0.4% for fruits using maceration with chloroform. Changing process, yields E.O after extraction by steam distillation yield is almost zero. The inhibition of E.O tests on the growth of certain bacteria (*Pseudomonas aeruginosa* 7853, *Listeria monocytogenes*, *Escherichia coli* 27922, *Staphylococcus aureus* and *Bacillus subtilis* SP2VSS2LG 6633) were carried out by two qualitative methods. The results show the inhibitory effect of E.O this taxon effect on some bacterial strains.

Key words: *Chamaerops humilis*, antibacterial activity, bacteria, essential oils.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Chamaerops humilis L. (Palmetto) of its common name "Doum" is a monocot of the Arecaceae family. It is a west mediterranean species [1]. In Algeria this taxon is widespread in western Algeria [2]. This species is used in traditional medicine to treat many ailments mainly those affecting the digestive tract [3 - 6]. Many studies have been done on E.O and their effect on bacterial inhibition [7, 8]. Therefore, the E.O is used in alternative medicine for a very long time for their antimicrobial properties. The antifungal and antibacterial activity of these secretions has been the subject of numerous in vitro studies [9 - 12].

This study was conducted to know the effect of E.O extracted from the aerial parts of the plant, mainly the leaves and fruit of some pathogenic strains. No work has been done to date of the effect of E.O on the antibacterial activity of this plant.

To better understand the antibacterial activity we selected reference strains namely *Pseudomonas aeruginosa* 7853, *Listeria monocytogenes* 15313, *Escherichia coli* 27922, *Staphylococcus aureus* SP2VSS2LG and *Bacillus subtilis* 6633.

MATERIALS AND METHODS

The plant material

The leaves and fruit of *Chamaerops humilis* were collected from subjects belonging to chamaeropaies located in the mountain ranges of Tlemcen (western Algeria) in September 2011. The plant was identified and authenticated by the botanist Dr Hasnaoui (University of Saida). The parts of the plant on which our work was done were cut into small pieces and dried at room temperature (20 ° C) and protected from light for two weeks. After drying the pieces obtained were ground and stored in sealed bottle until used.

Extraction of essential oils

The extraction (E) essential oil (E.O) was carried out by three methods: E. hydrodistillation (E.D), E. Soxhlet (E.S) and E. by maceration (E.M).

Extraction method

In the E.D and E.S we used 30 grams (g) of plant material. The techniques used are those described by Wenqiang et al, [13] and Ashnagar et al. [14]. The solvents used in this study vary depending on the process: it is the water in the E.D and hexane in the E.S. As for, we used 20 g of plant material in 60 ml of chloroform and 20 g in 50 ml of H_2O. In both methods it is based on the extraction methods described by Ashafa and Afolayan [15], Benmehdi et al. [16] and Gacem et al. [17].

Calculation of performance

The yield of essential oil (Y.E.O) is defined as the ratio of the E.O obtained after extraction mass (M ') and the mass of the plant material used (M) [18].

The yield is expressed as a percentage, and it is given by the following formula:

$$Y.E.O\ (\%) = M'/M \times 100$$

Y.E.O: essential oil yield of dry matter;
M '= mass in grams of essential oil from the dried plant material;
M: mass of the dried plant material used in grams.

Evaluation of antibacterial activity

Two reference methods were used:

- Technical agar diffusion Mueller Hinton (disc method or aromatogramme) [19] ;
- Method of micro-dilutions of liquid medium for the determination of minimum inhibitory concentration (MIC) [20].

Reading is done by measuring the diameters of the zones of inhibition around the disk according to international standards [21 - 24].

RESULTS AND INTERPRETATIONS

The yield of essential oils (Y.E.O)

The Y.E.O varies according to the method used. The yield obtained using E.S is 5% for the leaves while it is only 2.7% for fruits. The results obtained using the technique of E.M are low. The solvent used in E.M plays a significant role in the Y.E.O. The Y.E.O is 1.5% for leaves and only 0.4% for fruit on the E.M in H_2O results are low; they are respectively 0.3% and the percentage 0.2% through 2%. The E.H has given only a few traces in two parts used (Table I).

Table I: Change in Y.E.O depending on the method used.

	Extraction method	Quantity of material plant	The solvent	The extraction time (h)	Performance %
Leaves	E.S	30	Hexane	6h	5
	E.D	30	water	2h	Traces
	E.M	20	Chloroforme	15min	1.5
	E.M	20	water	15min	0.3
Fruits	E.S	30	Hexane	6h	2.7
	E.D	30	water	2h	Traces
	E.M	20	Chloroforme	15min	0.4
	E.M	20	water	15min	0.2

The results have different values for the two parts of the plant. The performance depends to a large part of the extraction method used. The E.S provides a significant performance compared to other methods (E.D and E.M). In quantitative terms the leaves are rich in E.O as fruit. E.M gives a lower result with 1.5% for leaves and 0.4% for fruits.

ISSN: 0975-8585

Antibacterial effect of E.O *Chamaerops humilis*

Determination of the minimum inhibitory concentration (M.I.C) by the method of micro-dilutions of liquid medium

The results of the antibacterial effect by micro-dilution method show that E.O has an inhibitory effect at a concentration of about 250 mg / ml for bacteria: *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli*. The inhibitory concentration is about 500 mg / ml for *Listeria monocytogenes* and *Bacillus subtilis* (Table II).

Table II: E.O inhibitory concentration.

	1	2	3	4	5	6	7	8	9	10	11	12
Staphylococcus aureus	-	-	+	+	+	+	+	+	+	+	+	+
Pseudomonas aeruginosa	-	-	+	+	+	+	+	+	+	+	+	+
Listeria monocytogenes	-	+	+	+	+	+	+	+	+	+	+	+
Echerichia coli	-	-	+	+	+	+	+	+	+	+	+	+
Baccilus subtilis	-	+	+	+	+	+	+	+	+	+	+	+
CMI(mg/ml)	500	250	125	62.5	31.25	15.62	7.81	3.9	1.95	0.97	0.48	-

Plus (+) = not inhibitory concentration; Minus (-) = inhibitory concentration

Technical agar diffusion Muëller Hinton (disc method) « Aromatogram »

The table 3 shows an antibacterial activity for three strains at a concentration 3V/3V. The diameters of inhibition zone is various, it is 2mm for *S. aureus, E. coli, P. aeruginosa* and 1mm of 2V/3V for *S. aureus*. In other cases no activity has been reported (Table III, Photo 1). These results show that E.O of *Chamaerops humilis* has a spectrum of antibacterial activity against Gram + and Gram -.

Table III: Antibacterial activity of E.O oils according to the method of discs.

	V/3V	**2V/3V**	**3V/3V**
Staphylococcus aureus (G+)	0	1mm	2mm
Baccilus subtilis (G+)	0	0	0
Listeria monocytogenes (G+)	0	0	0
Escherichia coli (G-)	0	0	2mm
Pseudomonas aeruginosa (G-)	0	0	2mm

The research for new drugs using several approaches includes the "ethno-pharmacological" approach, which is to gather information about the empirical use among people, ethnicities still living close to nature [25]. The E.O is used in alternative medicine for a very long time for their antimicrobial properties. The antifungal and antibacterial activity of these secretions has been the subject of numerous in vitro studies [9, 10, 12].

ISSN: 0975-8585

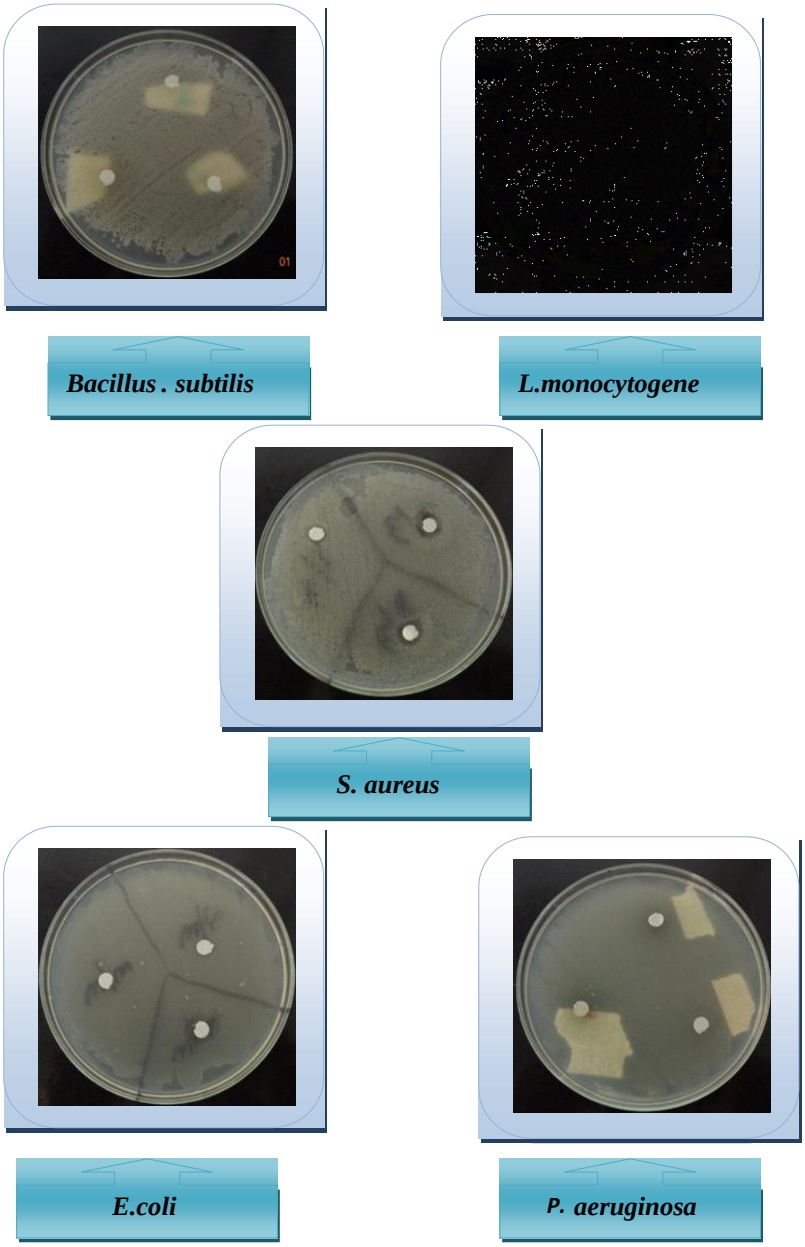

Photo 1: Antibacterial power of leaves of *Chamaerops humilis*.

The antimicrobial effect of E.O on two parts of *Chamaerops humilis* on some strains of bacteria gave interesting results. Indeed, the various analyzes carried out by Hasnaoui et al. [5, 6] on the most used parts of *Chamaerops humilis* show chemical groups present in the plant such as: saponins, tannins, alkaloids, flavonoids (free flavonoids heteroside and Flavonoids glucoside), steroids, sterols and unsaturated terpenes. These differents groupes are the main groups that enter into the constitution of the parties analyzed. The presence of these chemical groups indicates the medicinal use of *Chamaerops humilis*. Some flavonoids are anti-inflammatory drugs [26], antiviral, anti-tumor, anti-hypertensives and diuretics [27] and anti carcinogenic [28]. In addition, flavonoids may prevent atherosclerosis and thus reduce the risk of cardiovascular disease [28].The evaluation of the inhibitory activity of bacteria by two methods: Aromatherapy program and micro-dilution liquid medium indicated the MICs of E.O for bacteria are of the order of 250 mg / ml. In our case we found that the dilution method tells us more about the antibacterial diffusion method or disk activity.

Nevertheless, the antibacterial activity can be influenced by certain factors: in the first one, the absence of relation between both methods of study lies in the environment where are these extracts; if with the diffusion method certain zones of inhibition are very wide it is because the active constituents are free and have no direct contact with the constituents of the culture medium; while with the method of dilution the active constituents react with the ingredients of the culture medium and consequently can be inhibited or blocked.

It should be noted the significant effect exerted by organic materials when present in the environment: they are likely to significantly reduce the effectiveness of an antiseptic agent by combining with it to form inactive compounds, the adsorbent and decreasing its concentration or by precipitating and removing altogether [29]. Thus, the antibacterial activity may depend on the composition of the culture medium [25], in the case of Ail, for example, that garlic has a powerful in vivo when power is traditionally used against respiratory diseases.The presence of low bacterial growth with alternative inhibition is due to resistant bacterial strains and was able to grow in the presence of the antibacterial agent who is in conflict with it, or the instability of the extract. It is known that all species do not have the same sensitivity to a substance and in opposite a bacterial population, there may be individual differences in sensitivity [29]. Thus, the antibacterial action is sometimes partial and after a decrease in the number of bacteria, it is observed an increase of bacterial growth. This phenomenon called rebound may be due to instability of the in vitro antibacterial agent at a heterogeneity of the bacterial population may contain genotypically more resistant bacteria than the general population or induction of enzymes conferring bacterial resistance to antibacterial extract [30].

Some authors reported a relationship between the structure and antibacterial activity of the molecules that make up E.O in the presence of certain functional groups. The aldehyde and phenolic compounds are very active in clearly defined [31]. The Phenolic structures are the most active in other organisms, the presence of the hydroxyl group and its position, aldehydes also have potent antimicrobial activity, the presence of oxygen in ketones increases antimicrobial properties of the terpenes.

CONCLUSION

The results obtained in these analyzes reveal different yields of the E.O, this is a function of the part of the plant used. The leaves are rich in E.O, by against fruits are low in E.O. It should also be noted that the yields obtained Y.E.O change with the extraction process used. In terms of antibacterial activity we observed a variable inhibition. The most sensitive bacteria in our study are as follows: *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*.

REFERENCES

[1] Quézel P et S. Santa, 1962 ; Nouvelle de l'Algérie et des régions désertiques méridionales, Edit. C.N.R.S, T. 1, p 178.

[2] Hasnaoui 0. 2008 ; Contribution à l'étude des Chamaeropaies dans la région de Tlemcen: Aspects écologique et Cartographie. Université Abou Bakr Belkaid, Tlemcen, Algérie, Thèse Doct. 203 p. + annexe.
[3] Bnouham M, Mekhfi H, Legssyer A & Ziyya A. Int J Diabetes & Metabolism 2002;10: 33-50.
[4] Gaamoussi F, Israili Z, and Lyoussi B. Pak J Pharm Sci 2010 ;23(2)212-219.
[5] Hasnaoui Okkacha, Bouazza Mohamed et Benali Omar. 2011 ; Impact socio-économique du *Chamaerops humilis* L. dans la société Algérienne Annales de l'Université de Bechar N° 9, 2011, 9 ISSN : 1112-6604
[6] Hasnaoui O, Benali O, Bouazza M and Benmehdi H. Res J Pharm Biol Chem Sci 2013;4(2):910-918.
[7] Ghalem BR and Mohamed B. African J Pharm Pharmacol 2008;2(10);211-215.
[8] Morais-Braga MF, Souza TM, Santos KA et al. Boletin Latinoamericano y del Caribe de Plantas Medicinales y Aromaticas 2013 ;12(1): 38-42.
[9] Trivedi NA and Hotchandani SC. Indian J Pharmacol 2004 ; 36: 93-94
[10] Giordani R et Kaloustian J. 2006 ; Action anticandidosique des huiles essentielles: Leur utilisation concomitante avec des médicaments antifongiques. 121-124, DOI: 10.1007/s10298-006-0165-7.
[11] Al-Bayati FA. J.Ethnopharmacol 2008;116(28): 403-406.
[12] Nair R, Vaghasiya Y and Chanda S. Afr J Biotechnol 2008;7:25-26
[13] Wenqiang G, Shufen L, Ruixiang Y, Shaokun T and Can Q. 2006; Comparison of essential oils of clove buds extracted with supercritical carbon dioxide and other three traditional extraction methods; China.
[14] Ashnagar A, Gharib N and Haidari NH. E-J Chem 2007;4(4):546-549.
[15] Ashafa A. et Afolayan A. J Med Plants Res 2009;3(8:568-572.
[16] Benmehdi H, Hasnaoui O, Benali O and Salhi F. J Mater Environ Sci 2012;3(2):320-237.
[17] Gacem MA, Ould El Hadj Khelil Aminata, Hadef Sawsen, Boudarham Amel et Djerbaoui Amina Nasrine. PCBS J 2013 ;7(3):90-94.
[18] Bssaibis F, Gmira N. et Meziane M. Rev. Microbiol. Ind. San et Environn. 2009 ;3(1) : 44-55
[19] NCCLS. Performance Standards for antimicrobial disk susceptibility tests, 2006;(N°1): 48 Approved standards. Seventh Edition, Document NCCLS, M2-A7.
[20] CLSI (Clinical and Laboratory Standards Institute), 2002; Reference method for broth dilution antifungal susceptibility testing of yeasts, approved standard M27-A2, 2nd ed. Clinical and Laboratory Standards Institute, Villanova, PA, 22 (15).
[21] CLSI (Clinical and Laboratory Standards Institute), 2006; Methods for dilution antimicrobial susceptibility testing for bacteria that grow aerobically. Approved standard M7-A7.Clinical and Laboratory Standards Institute, Wayne, PA; 26 (2).
[22] Franchomme P. 1999. L'aromathérapie thérapeutique de pointe en médecine naturelle. Sinceiro – Entreprises Ltd, Hong Kong.
[23] Farah A, Satrani B, Fechtal M, Chaouch A. et Talbi M. Acta Bot Gallica 2001 ;148(3): 183-190.
[24] Cimanga K, Kambu K, Tona L, Apers S, De Brugne T, Hermans N, Totté J, Pieters L. and Vlietink A.J., J. Ethnoparmacol 2002;2(79): 213-220.
[25] Roulier G. Appl Environ Microbiol 1999 ;70 (10) 5750-55.

[26] Bidet D, Gaignault JC. et Trotin F. 1987, Inflammation, allergie, douleur et acide arachidonique : du jardin des Hespérides à la cascade de l'acide arachidonique : les flavonoïdes. L'actualité chimique, 89-97.
[27] Bruneton J, 1999, Pharmacognosie et phytochimie – plantes médicinales, technique et documentation. 3ème Ed. Lavoisier, Paris.
[28] Marfak A, 2003, Radiolyse gamma des flavonoïdes : Etude de leur réactivité avec les radicaux issus des alcools : formation de depsides ; Thèse de doctorat, Faculté de Limoges France. Médecine-Sciences Flammarion.
[29] Leclerc H, Gaillard J-L, Simonet M., 1995; Microbiologie générale, la bactérie et le monde bactérien. Doin Editeurs. Paris
[30] Berche P, Gaillard J-L, Simonet M., 1989; Bactériologie: bactéries des infections humaines.
[31] Friedman M, Henika PR and Mendrell RE. J Food Prot 2002 ;65 (10) 1545-1560.