IJIRR

International Journal of Information Research and Review
Vol. 04, Issue, 02, pp.3604-3610, February, 2017

Research Article

EVALUATION OF ANTIBACTERIAL AND HAEMOSTATIC ACTIVITIES OF ORGANIC EXTRACTS OF *CARDOPATIUM CORYMBOSUM* L

[1,*]Leila HAMBABA, [1]Ghania YAKHLEF, [1]Leila KARA, [1]Kelthoum BOUABDELLAH, [1]Nabil GHEDADBA, [2]Amar AYACHI and [3]Mohamed Cherif ABERKANE

1Laboratory of Chemistry of materials and living: activity and reactivity, University, Batna1, 05000, Algeria
2Laboratory of microbiology, Institute of agronomy, University, Batna1, 05000, Algeria
3Laboratory of phytochemistry, Faculty of Sciences, University Batna1, 05000, Algeria

ARTICLE INFO

Article History:

Received 17th November, 2016
Received in revised form
20th December, 2016
Accepted 24th January, 2017
Published online February, 28th 2017

Keywords:

Cardopatium Corymbosum L.
Polyphenols,
Flavonoids,
Thin Layer Chromatography,
Antibacterial Activity,
Haemostatic Activity.

ABSTRACT

The first biological study of *Cardopatium corymbosum* L. consists of analyzing qualitatively and quantitatively the methanolic, dichloromethanic, and etheropetrolic extracts from aerial part and investigating the antibacterial and haemostatic activities. The qualitative analyses of these extracts by thin layer chromatography (TLC) reveal the probable presence of quercetin and 4- hydroxybenzoic acid in the methanolic extract. The quantitative estimation of total polyphenols (by Folin-Ciocalteu's method), the flavonoids (by $AlCl_3$ method), and condensed tannins (by vanillin method) has shown the richness of these extracts. The extracts are also subjected to a sifting for their possible antibacterial activity *in vitro* against eight species of bacteria by employing Agar-gel diffusion method. Each one is reacted positively on one of the bacterial strains tested at least. The evaluation of haemostatic activity (by the method of recalcification of plasma) showed a diminution of coagulation tense.

INTRODUCTION

Traditionally, medicinal plants have been used to treat all kinds of human diseases (Bauer *et al*., 2003). There is an urgent need to discover new antimicrobial compounds with novel mechanisms of action due to an alarming increase in the incidence of new and re-emerging infectious diseases and development of resistance to the antibiotics in current clinical use (Dimayuga, 1991). The screening of plant extracts is of great interest to scientists in the search for new drugs for greater effective treatment of several maladies (Diallo *et al*., 1999). The main sources of bioactivity in medicinal plants are the plant secondary metabolites they produce (Erdogrul, 2002). The Asteraceae is the second largest family in the Magnoliophyta Division with around 1100 genera and over 20000 recognized species (Cabrera, 1978). One of the members of the Asteraceae family is *Cardopatium corymbosum* L. (Barres *et al*., 2013) (known as *Carthamus corymbosus*) (Quezel, 1963).

****Corresponding author: Leila HAMBABA,***
Laboratory of Chemistry of materials and living: activity and reactivity, University, Batna1, 05000, Algeria.

It is largely known in Cyprus as antiseptic (Pieroni *et al*., 2006; Lardos, 2006) and for the treatment of digestive diseases (Gonzalez-Tejero *et al*., 2008). The goal of this study is to evaluate the antibacterial activity by diffusion in gelose medium method and the haemostatic activity by the method of recalcification of plasma.

MATERIALS AND METHODS

Plant material and extracts preparation

Aerial parts of *Cardopatium corymbosum* were freshly collected in summer season from Bouhmama, Khenchela, Algeria in May 2012. The taxonomic identification of the plant was confirmed by Pr. Oudjhih B., institute of Agronomy, University of Batna. The plant was dried in the shade under ambient temperature until total dehydratation. Dried aerial parts were blended and stored in the dark at a dry place. 200 g of powder was extracted with 2 L of petroleum ether, then the marc was extracted with 2 L of dichloromethane and finally with 2 L of methanol.

The extracts were recuperated by evaporating the solvent under controlled temperature (35º, 40º, and 45°C respectively)(Biallo *et al*., 2004).

Preliminary phytochemical analysis

The different extracts were then subjected to preliminary phytochemical analysis to assess the presence of various phytoconstituents.

Determination of total phenolic compounds, total flavonoids, and tannins

Determination of total phenolic compounds

Content of polyphenols in the extracts is estimated and carried out according to the process described by Singleton and *al* (12). The phenolic contents were given with the method of Folin-Ciocalteu. To 0.2 ml of each extract (prepared in methanol at a known concentration) is added 0.8 ml of Na_2CO_3 solution (75 mg/ml), after agitation, 1 ml of Folin-Ciocalteu solution (diluted ten times in distillated water) is inserted. The mixture was incubated at ambient temperature during 2 hours. Lastly, absorbance was measured with the spectrophotometer assistance at 765 nm. The concentration of total polyphenols in our extracts was calculated starting from a linear calibration curve (y=ax+b) established with precise concentrations of gallic acid (0-200 µg/ml) like standard of reference, under the same conditions as the sample. The total phenolic contents were expressed in mg EAG/g of sample.

Determination of total flavonoids

The flavonoids were estimated by using the method of Yi and *al.* (2007). 1ml of methanolic solution of $AlCl_3$ of 2 % was added to 1 ml of the extract. After 10 min of incubation at ambient temperature, the absorbance is measured at 430 nm. Content of flavonoids is expressed by milligram of equivalent of quercetin per gram of extract (mg EqQ/g of extract) starting from a calibration curve established by quercetin (0-40 µg/ml).

Determination of condensed tannins

The proportioning of condensed tannins in the extract is carried out according to the method of Hagerman (Hagerman, 2002) with some changes. The principle of this proportioning is based on the reaction of the vanillin with the free flavan-3-ols and the terminals unities of pro-anthocyanidins to form a red chromophoric complex which is detected at 500 nm.

Operatory mode

- ✓ Two (02) chains of tubes are prepared.
- ✓ One (01) ml of extract is introduced in one tube of each of the tow chains.
- ✓ Five (05) ml of analysis reactif (vanillin 2 % - HCl 8 % in methanol V/V)is added to the tubes of the first chain.
- ✓ Five (05) ml of HCl 4 % is added to the tubes of the second chain.
- ✓ The tubes are incubated at 30 °C during 20 minutes.
- ✓ The absorbance of tubes of the second chain is subtracted from the one of the first chain because the blanc can be considerable for the tissues which contain an important quantity of pigments. The obtained values are used to deduce the concentration in condensed tannins. Content of condensed tannins is expressed by milligram of equivalent of catechin per gram of extract (mg EqC/g of extract) starting from a calibration curve established by catechin (0 - 400 µg/ml).

Qualitative analysis

Characterization by thin layer chromatography (TLC)

The analysis were effected in normal phase, with silica plaque (Silicagel 60A, of 0.25 mm of thickness) putted on nylon paper which constitute the stationary phase. 10 µl of the extract (40 mg/ml) and the standards (2 mg/ml) were put on the plaque. The mobile phase was constituted from Chloroform-Ethyl acetate- Formic acid (60:35:5 V/V/V) for the methanolic extract and Petroleum ether- Ethyl acetate (80: 20 V/V) for dichloromethanic and etheropetrolic extracts.

After development, the TLC plaque were dried and observed under UV at 254 nm and 365 nm, sprayed by sulfuric vanillin mixture (Godin reactive), then dried at 60ºC during 5 minutes to reveal the spots stem from separation.

Biological activities tests

Antibacterial activity

Germs tested to detect the antibacterial activity of the extract were three strains of reference: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and five clinical strains: *S. epidermidis*, *S.* sp., *Enterobacter* sp., *Proteus vulgaris*, *Klebsiella pneumonia*. There were all provided by the laboratory of Bacteriology from the University Hospital Center of BATNA.

Method of diffusion in medium gelose

Sterile filter paper discs of 6 mm of diameter, impregnated into 10 µl of extract (15), were placed on the surface of the inoculated agar plates. The extract was taken with dimethyl sulfoxide (DMSO). The antibacterial screening was carried out with different concentrations (1 g/ml, 500 mg/ml, 250 mg/ml, 125 mg/ml, and 62.5 mg/ml) except etheropetrolic extract with concentrations of 500 mg/ml, 250 mg/ml, and 125 mg/ml. Controls soaked in DMSO were only performed. Petri dishes were incubated for 24 h at 37ºC. Antibacterial activity was determined by measuring the diameter of the inhibition's zone.

Haemostatic activity test

This test is more sensible than the entire blood one. The principle of this test consist to measure the coagulation tense of decalcified plasma after recalcification (Borel *et al*., 2004). The methanolic extract has been prepared with distillated water to obtain different dilutions (40, 20, and 10 mg/ml). The blood samples were collected on citrate of sodium from healthy subjects, the plasma is obtained by centrifugation at 3600 rpm during 10 mn.

Study protocol

- Distribution of methanolic extract solutions at a rate of 50, 100, 200 µl in hemolytic tube for each dose.
- An empty tube has been used as witness.
- The tubes were placed in water-bath at 37ºC
- Insert in each tube (hemolytic tube and empty tube) 0.2 ml of plasma and 0.2 ml of chloride of calcium (0.025 M), triggering in the same time of stopwatch.
- Slope each tube at an angle of 45º until the observation of blood clot, note the coagulation tense in the two tube of each dose.

Statistical study

The analysis of variance has been realized by Graph Pad Prism V 5.00. The correlation tests were carried out to determine the relation between the content of phenolic compounds of the extracts and the studied activities. They are considered significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

Yield of extraction

The extraction method allow to obtain three crude extracts: petroleum ether extract (EEP), dichloromethane extract (EDCM), methanolic extract (EMeOH). The yields of extraction are mentioned in the table bellow (Table 1).

Table 1. Aspect, color, and yield of extracts of *Cardopatium corymbosum* L

Extract	Aspect	Color	Yield (%)
EEP	Oiled paste	Green	1.350
EDCM	Sticky paste	Black	0.795
EMeOH	Doughy	Brown	2.150

The calculation of yields compared with whole weight of the used powder showed that the plant give weights higher than 1g/ 100g of powder plant of methanolic and etheropetrolic extracts, while the weight is lower than 1 g/100g of powder for dichloromethane extract.

Preliminary phytochemical analysis

Total phenolic compounds, flavonoids, and condensed tannins

Table 2. Total phenolic compounds, flavonoids, and condensed tannins in various extracts of *Cardopatium corymbosum* L

extract	Polyphenols [(a)]	Flavonoids [(b)]	Condensed tannins [(c)]
EEP	3.562±0.479	0.657±0.44	0.005±0
EDCM	7.277±1.166	3.905±1.52	0.011±0.001
EMeOH	7.754±0.235	2.379±0.60	0.010±0.003

[(a)]: milligram equivalent of gallic acid per gram of extract.
[(b)]: milligram equivalent of quercetin per gram of extract.
[(c)]: milligram equivalent of catechine per gram of extract.

Table 2 total phenolic compounds, flavonoids, and condensed tannins in various extracts of *Cardopatium corymbosum* L. Several factors can influence the content of phenolic compounds, recent studies showed that geographical and climatic factors, temperature, fertility (Kim *et al*., 2004 ; Wojdylo *et al*., 2007) salinity, drought (Falleh *et al*., 2008), genetic factors, but also the degree of maturation of the plant and storage period have a strong influence on the polyphenols content.

The concentration of flavonoids in the plant's extracts is differed according to the extraction method (Wojdylo *et al*., 2007) and to the polarity of solvents used in the preparation of the extracts.

Characterization by thin layer chromatography (TLC)

The use of TLC reveals the richness of various extracts of *Cardopatium corymbosum* where we identify the presence of 14 marks for EEP, 13 marks for EDCM, and 14 marks for EMeOH. Petroleum ether and dichloromethane solvents are generally used to skim the drug. With the naked eye, spots with Rf equal 0.09, 0.25, 0.94, 0.07, and 0.94 appear green which signify that are typically chlorophylls. Rutin did not migrate in the used solvents system. By the calculation of Rf of EMeOH and in comparison with standards Rf, we identify the presence of:

- 4-hydroxybenzoic acid;
- Quercetin.

The difference of color between spots stem from the separation of extracts and corresponding standards due to the effect of mixture because a pure component has a different comportment in comparison with the same component in the extract.

Biological activities

Antibacterial activity

The results of antibacterial activity of aerial part of *C. corymbosum* are positive (Table V, VI, and VII). All the extracts of C. corymbosum are revealed actives with different degrees. For the methanolic extract, *Proteus vulgaris* is the most sensible one with 11.77 mm just as EDCM with 13.25 mm. The content of total polyphenols has been show a very significant correlation with the antibacterial activity of *Staphylococcus sp.* with R^2=0.94 and P=0.002 (fig. 1). Fig.1: correlation between total polyphenols of dichloromethanic extract and *S. sp.* It appears that Staphylococcus sp. Gram + is the most likely bacterium by comparison with the other Gram strains, this can be allotted to the difference of the structure between the bacteria Gram + and the bacteria Gram – (Koné *et al*., 2004; Turkmen *et al*., 2007 ; Shan *et al*., 2007; Hayouni *et al*., 2007). This antibacterial activity can be due to the synergistic action of polyphenols (Essawi, 2000). They revealed that certain chemical components like tannins and flavonoids are used as defense's mechanisms of many micro-organisms and the antibacterial activity of flavonoids is probably done to their capacity to be complexed with extracellular proteins of the bacterial cellular wall (Doss *et al*., 2011). It appears also that the method used to evaluate the antibacterial activity has a great influence (Natarajan *et al*., 2005; Fazeli *et al*., 2007).

Haemostatic activity

The haemostatic activity of the methanolic extract is evaluated with recalcification plasma test according to the method described by Borel *et al.* (1984). The average percentages of diminution of coagulation tense of methanolic extract for growing concentrations: 10, 20, and 40 mg/ml are respectively 18.35, 27.07, and 28.64 % which mean that the diminution of coagulation tense is dose dependent.

Table 3. Results of separation of EEP and EDCM by TLC

Extract	N°spot	*Rf*	Observation at 254 nm	Observation at 365 nm	Godin
	EP_1	0.07	Visible	Visible	Green
	EP_2	0.19	-	Visible	Grey
	EP_3	0.25	Visible	Visible	Violet
	EP_4	0.34	-	Visible	Brown
	EP_5	0.42	Visible	Visible	Blue
	EP_6	0.46	Visible	Visible	Pinky
EEP	EP_7	0.53	Visible	Visible	Blue
	EP_8	0.59	-	-	Brown
	EP_9	0.69	Visible	-	Violet
	EP_{10}	0.74	Visible	-	Green
	EP_{11}	0.82	Visible	-	Violet
	EP_{12}	0.88	Visible	-	Green
	EP_{13}	0.94	Visible	-	Green
	EP_{14}	0.98	Visible	-	Green
	DCM_1	0.09	Visible	Visible	Green
	DCM_2	0.14	-	Visible	Green
	DCM_3	0.25	-	Visible	Green
	DCM_4	0.36	-	Visible	Brown
	DCM_5	0.43	Visible	Visible	Blue
	DCM_6	0.54	Visible	Visible	Blue
EDCM	DCM_7	0.60	Visible	Visible	Brown
	DCM_8	0.69	Visible	Visible	Violet
	DCM_9	0.75	-	-	Green
	DCM_{10}	0.82	-	-	Violet
	DCM_{11}	0.89	-	-	Green
	DCM_{12}	0.94	-	-	Green
	DCM_{13}	0.98	Visible	-	Green

standards	*Rf*	Observation at 254 nm	Observation at 365 nm	Godin
Rutine	-	-	-	-
Acide gallique	0.15	Violet	-	Reddish
Acide trancinnamique	0.78	Violet	-	-
A.hydroxybenzoique			-	-
A.tannique	0.60	Violet		
Quercétine	0.21	Violet	Violet	-
	0.50	Orange	Brown	Orange

Table 4. Results of separation of EMeOH with TLC

Extract	N°spot	*Rf*	Observation at 254 nm	Observation at 365 nm	Godin
	$MeOH_1$	0.06	Visible	-	Green
	$MeOH_2$	0.10	-	-	Brown
	$MeOH_3$	0.18	Visible	-	Violet
	$MeOH_4$	0.26	-	-	Violet
	$MeOH_5$	0.30	-	-	Yellow
	$MeOH_6$	0.42	-	-	Violet
EMeOH	$MeOH_7$	0.50	-	-	Brown
	$MeOH_8$	0.56	Visible	-	Reddish
	$MeOH_9$	0.60	-	-	Green
	$MeOH_{10}$	0.77	-	-	Violet
	$MeOH_{11}$	0.82	-	-	Violet
	$MeOH_{12}$	0.87	-	-	Violet
	$MeOH_{13}$	0.91	-	-	Violet
	$MeOH_{14}$	0.95	Visible	-	Green

Table 5. Diameter of inhibition zone obtained with EEP.

	500 mg/ml	250 mg/ml	125 mg/ml
Staphylococcus spp.	9.59±1.70	9.38±1.72	7.83±1.32
Staphylococcus épidermidis	-	-	-
Staphylococcus aureus	8.88±0.86	8.83±0.51	8.17±0.94
Klebsiella pneumonia	-	-	-
Escherichia coli	8.28±0.35	8.06±0.21	-
Proteus vulgaris	-	-	-
Pseudomonas aerugenosa	-	-	-
Entérobacter sp.	-	-	-

a : chromatogram photograph under UV at 254 nm.
b : chromatogram photograph under UV à 365 nm.
c : chromatogram photograph after revelation with Godin reactive.

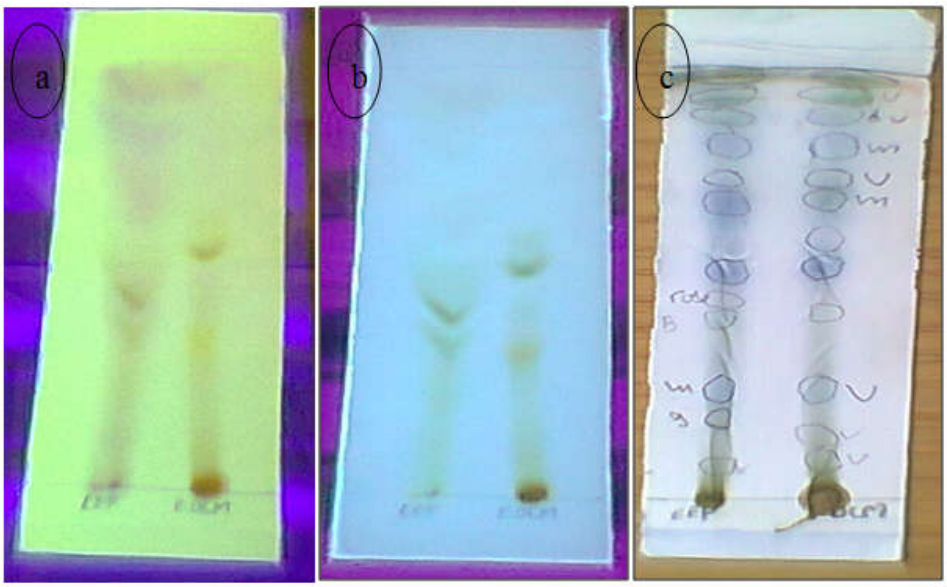

Fig. 1. chromatograms of EEP and EDCM extracts

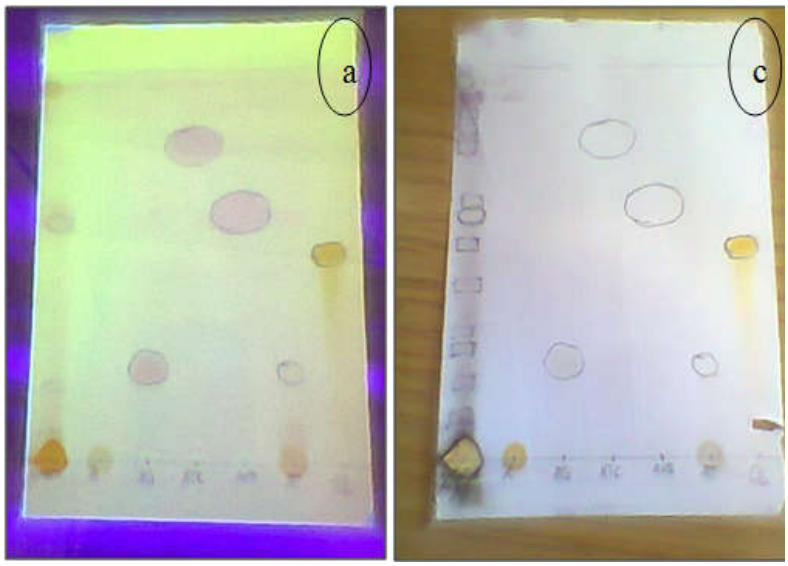

Fig. 2. Chromatograms EMeOH extract

Table 6. Diameter of inhibition zone obtained with EDCM.

	1 g/ml	500 mg/ml	250 mg/ml	125 mg/ml	62.5 mg/ml
Staphylococcus sp.	8.41±0.38	7.50±0.38	7.41±0.26	7.30±0.42	7.03±0.21
Staphylococcus épidermidis	-	-	-	-	-
Staphylococcus aureus	9.07±0.09	-	-	-	-
Klebsiella pneumonia	-	-	-	-	-
Escherichia coli	8.62±0.21	-	-	-	-
Proteus vulgaris	13.25±1.13	7.54±0.24	6.90±0.10	-	-
Pseudomonas eruginosa	-	-	-	-	-
Entérobacter sp.	-	-	-	-	-

Table 7. Diameter of inhibition zone obtained with EMeOH.

	1 g/ml	500 mg/ml	250 mg/ml	125 mg/ml	62.5 mg/ml
Staphylococcus sp.	8.04±0.66	7.71±1.13	7.53±0.40	7.52±0.86	7.33±0.36
Staphylococcus épidermidis	-	-	-	-	-
Staphylococcus aureus	-	-	-	-	-
Klebsiella pneumonia	7.09±0.07	-	-	-	-
Escherichia coli	-	-	-	-	-
Proteus vulgaris	11.77±0.14	10.04±0.05	-	-	-
Pseudomonas aeruginosa	-	-	-	-	-
Entérobacter sp.	-	-	-	-	-

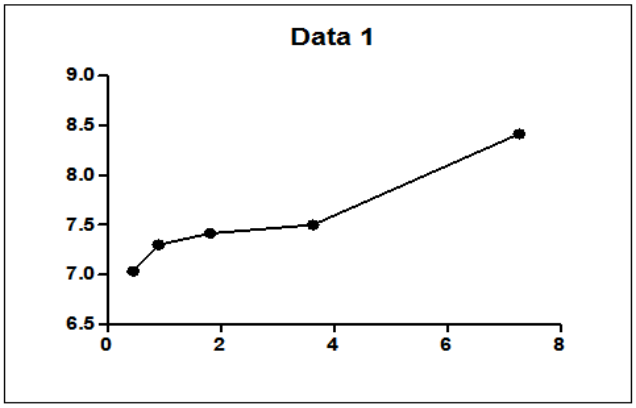

Fig.1. correlation between total polyphenols of dichloromethanic extract and *S. sp*

Table 8. Average Percentages of diminution of coagulation tense

Concentration de l'extrait(en mg/ml)	Pourcentages moyens (%)
10	18.35±0.19
20	27.07±0.10
40	28.64±0.15

These results appear logic because the methanolic extract contains tannins which are known by their coagulant effect (Daas Amiour, 2009; Özacar, 2002). According to El-kalamouni (El kalamouni, 2010), the haemostatic activity of the Asteraceae family is largely known such as the use of *Achillea mellifolium* in the treatment of hemorrhoids.

Conclusion

The thin layer chromatography allows revealing the presence of many chemical compounds such as the possible presence of 4-hydroxybenzoic acid and quercetin in methanolic extract. The quantitative estimation of total polyphenols by Folin Ciocalteu's method showed that the polar extract content the highest value with 7.75±0.23 mg EqAG/g of extract, when the quantification of flavonoids by trichlorure of aluminum method and condensed tannins with vanillin method showed that the EDCM content the highest values with 3.90±1.52 mg EqQ/g of extract and 0.011 mg EqC/g of extract respectively. The evaluation of antibacterial activity showed that all the extracts react positively on one of the bacterial strains tested at least where *Proteus vulgaris* was the most sensible bacterium with 13.25 mm. The haemostatic activity tested with plasma's re-calcification method with the methanolic extract showed a diminution in coagulation tense which signify the presence of an haemostatic activity.

REFERENCES

Barres, L., Sanmartin, I., Anderson, C.L., Alsonso, S., Buerki, S., Galbany-Casals, M. et Vilatersana, R. 2013. Reconstructing the evolution and bibliographic history of tribe Cardueae (compositae). *American Journal of Botany* 100(5): 000-000.

Bauer, J., Rojas, R., Bustamante, B. 2003. Antimicrobial activity of selected Peruvian medicinal plants. *J Ethnopharmacol,* 88: 199- 204.

Biallo, D., Sanogo, R., Yasambou, H. et autre. 2004. Étude des constituants des feuilles de *Ziziphus mauritiana* Lam. (Rhamnaceae). *C. R. Chimie.* 7 : 1073-1080

Borel, J., Caron, J., Chanard, J., Goureon, J., Leutenegger, M., Maquart, F.X., Potron, G., Randoux, A., Zeitoun, P. 1984. Comment prescrire et interpréter un examen de biochimie. 2eme édition. Maloine S.A. Editeur. Paris, 850 p.

Cabrera, A. L. Flora de la Provincia de Jujuy. ColecciónCientíficadel INTA. Buenos Aires, Argentina, 1978, pp. 311-318.).

Daas Amiour S. 2009. Etude quantitative des composés phénolique des extraits de trois variétés de dattes (*phoenix dactylifera* L.) et évolution in vitro de leur activité biologique. Thèse de magistère-Batna.

Diallo, D., Hveem, B., Mahmoud, M.A., Betge, G., Paulsen, B.S., Maiga, A. 1999. An ethnobotanical survey of herbal drugs of Gourma district, Mali. *Pharm Biol.,* 37: 80-91.

Dimayuga, R.E., Garcia, S.K.1991. Antimicrobial, screening of medicinal plants from Baja California sur, Mexico. *J Ethnopharmacol,* 31: 181-192.

Doss A., Vijayasanthi M., Parivuguna V., Venkataswami R., Antimicrobial effects of the flavonoid fractions of *Mimosa pudica* L. leaves. J Pharmacy Res, 4:5 (2011) 1438-1439.

El kalamouni C. 2010. Caractérisations chimiques et biologiques d'extraits de plantes aromatiques oubliées de Midi-Pyrénées. Thèse de doctorat. Toulouse.

Erdogrul, O.T. 2002. Antibacterial activities of some plant extracts used in folk medicine. *Pharm Biol.,* 40: 269-273).

Essawi T. et Srour M. 2000. Screening of some Palestinian medicinal plants for antibacterial activity. *J. Ethnopharm.* 70 : 343-349.

Falleh, H., Ksouri, R., Chaieb, K., Karray-Bouraoui, N., Trabelsi, N., Boulaaba, M. et Abdelly, C. 2008. Phenolic composition of *Cynara cardunculus* L. organs, and their biological activities. *C. R. Biologies*, 331, pp 372-379.

Fazeli, M. R., Amin, G., Ahmadian-Attari, M. M., Ashtiani, H., Jamalifar, H., Samadi, N. 2007. Antimicrobial activities of Iranian sumac and avishan-e shirazi (*Zataria multiflora*) against some food-borne bacteria. Food Control 18: 646-649.

Gonzalez-Tejero, M.R., Casares-Porcel, M., Sanchez-Rojas, C.P., Ramiro-Gutierrez, J.M., Molero-Mesa, J., Pieroni, A., Giusti, M.E., Censorii, E., De Pasquale, C., Della A., Paraskeva-Hadijchambi, D., Hadjichambis, A., Houmani, Z., El-Demerdash, M., El-Zayat, M., Hmamouchi, M., ElJohrig, S. 2008. Medicinal plants in the Mediterranean area: Synthesis of the results of the project Rubia. *Journal of Ethnopharmacology*. 116: 341–357.

Hagerman, A.E. 2002. Tannin Handbook. 2ème édition. Miami University. Oxford, USA, p116.

Hayouni, E.A, Abedrabba, M., Bouix, M., Hamdi, M. 2007. The effects of solvents and extraction method on the phenolic contents and biological activities *in vitro* of Tunisian *Quercus coccifera* L. and *Juniperus phoenicea* L. fruit extracts. *Food Chem*. (in press).

Kim, P. H., Son, H. K., Chang, W. H., et Kang, S. S. 2004. Anti-inflammatory Plant Flavonoids and Cellular Action Mechanisms. *Journal of pharmacological sciences*, 96: 229-245.

Koné, W.M., Kamanzi Atindehou, K., Terreaux, C., Hostettmann, K., Traoré, D., Dosso, M. 2004. Traditional medicine in North Côte-d'Ivoire: screening of 50 medicinal

plants for antibacterial activity. *J Ethnopharmacol*. 93: 43-49.

Lardos, A. 2006. The botanical *materia medica* of the *Iatrosophikon*—A collection of prescriptions from a monastery in Cyprus. Journal of Ethnopharmacology 104 (2006) 387–406.

Natarajan D., John Britto S., Srinivasan K., Nagamurugan N., Mohanasundari C., Perumal G. 2005. Anti-bacterial activity of Euphorbia fusiformis-A rare medicinal herb. *J Ethnopharmacol*. 102: 123-126.

Ngameni, B., Kuete, V., Simo I.K., Mbaveng, A.T., Awoussong, P.K., Patnam, R., Roy, R., Ngadjui, B.T. 2009. Antibacterial and antifungal activities of the crude extract and compounds from *Dorstenia turbinata* (Moraceae). *South African J Botany*. 75 : 256-261.

Özacar M., Sengil I A. 2002. The Use of Tannins from Turkish Acorns (Valonia) in Water Treatment as a Coagulant and Coagulant Aid. *Turkish J. Eng. Env. Sci*., 26 : 255 - 263.

Pieroni, A., Giusti, M.E., De Pasquale, C., Lenzarini, C., Censorii, E., Gonzáles-Tejero, M.R., Sánchez-Rojas, C.P., Ramiro-Gutiérrez, J.M., Skoula, M., Johnson, C., Sarpaki, A., Della, A., Paraskeva-Hadijchambi, D., Hadjichambis, A., Hmamouchi, M., El-Jorhi, S., El-Demerdash M., El-Zayat M., Al-Shahaby O., Houmani Z. and Scherazed, M. 2006. Circum-Mediterranean cultural heritage and medicinal plant uses in traditional animal healthcare: a field survey in eight selected areas within the RUBIA project. *Journal of Ethnobiology and Ethnomedicine.*2:16.

Quezel P. et Santa S. 1963. Nouvelle flore de l'Algérie et des régions désertiques méridionales. Ed de centre national de la recherche scientifique Paris-France.

Shan, B., Cai, Y.Z., Brooks, J.D., Corke, H. 2007. The *in vitro* antibacterial activity of dietary spice and medicinal herb extracts. *International J Food Microbiology*. 117: 112-119.

Singletonm V. L., Orthoferm R., Lamuela-Raventosm R. M. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin Ciocalteu reagent. *Methods in Enzymology.* 299 : 152-178.

Turkmen, N., Velioglu, Y. S, Sari, F., et Polat, G. 2007. Effect of Extraction Conditions on Measured Total Polyphenol Contents and Antioxidant and Antibacterial Activities of *Black Tea*. *Molecules*, 12: 484-496.

Wojdylo, A., Oszmianski, J., et Czemerys, R. 2007. Antioxidant activity and phenolic compounds in 32 selected herbs. *Food Chemistry*, 105, pp 940-949.

Yi, Z.B., Yu, Y., Liang, Y.Z., Zeng, B. 2007. In vitro antioxidant and antimicrobial activities of the extract of *Pericarpium Citri Reticulatae* of a new Citrus cultivar and its main flavonoids, *LWT-Food Science and Technology*. 4: 1000-1016.

****