Comparison of ultra-sound and maceration extraction methods of phenolics contents and antioxidant activities of Saharian...

Article *in* Journal of Materials and Environmental Science · May 2016

CITATIONS
0

READS
61

8 authors, including:

Chouikh Atef
El-Oued University
10 PUBLICATIONS **2** CITATIONS
SEE PROFILE

Hadia Hemmami
El-Oued University
6 PUBLICATIONS **1** CITATION
SEE PROFILE

Abdelkrim Rebiai
El-Oued University
33 PUBLICATIONS **73** CITATIONS
SEE PROFILE

J. Mater. Environ. Sci. 7 (6) (2016) 2235-2239
ISSN : 2028-2508
CODEN: JMESC

Comparison of ultra-sound and maceration extraction methods of phenolics contents and antioxidant activities of Saharian medicinal plant *Calligonum comosum* L'her.

A. Chouikh[1*], E.H. Adjal[1], M. Mekki[1], H. Hemmami[2], A. Feriani[3], A. Rebiai[2], A. Zaater[1], A. Chefrour[4,5]

[1]*Department of Biology, Faculty of Natural Science and Life, University of El Oued, BP 789 El-Oued (39000) Algeria.*
[2]*Laboratory Valorisation and Technology of Saharan Resources (VTRS).University of El Oued, BP 789 El-Oued (39000) Algeria.*
[3]*Lab of Ecophysiologie Animal, Faculty of Sciences, University of Sfax, Tunisia.*
[4]*Laboratory Development and Control of Hospital Pharmaceutical Preparations. Medical Science Faculty, University of Badji Mokhtar- Annaba, LP 205 Ezzafrania, Annaba (23000) Algeria.*
[5]*Faculty of Natural Sciences and life, University of Mohamed Cherif Messaadia-Souk Ahras (41000) Algeria.*

Received 14 Sep2015, Revised 26 Nov 2015, Accepted 08 Dec 2015
[*]Corresponding Authors. E-mail: atef-chouikh@univ-eloued.dz (Atef Chouikh); Phone: +213 666 68 47 15

Abstract
The aims of this study were to evaluate the effects of some extraction methods (Ultra-sound: UM, Maceration: MM) and solvents (Ethanol: EOH, Methanol: MOH) of the Polyphenols, flavonoid contents and antioxidant activities from *Calligonum comosum*. The EOH extracts contained high levels of Polyphenols and flavonoids when compared to MOH extracts. The ethanolic maceration method (EOH MM extract) had the highest antioxidant capacity (596.83 mg GAE/g Dried Extract). Also the EOH UM extract showed higher capacity (511.22 mg GAE/g Dried Extract), when compared to methanol extracts (275.41with MOH MM and 231.59mg GAE/g Dried Extract with MOH UM). The values of IC_{50} clearly indicated superiority of ethanol extracts (6.3 µg /ml for UM and 7.9µg/ml with MM) to methanol extracts (50µg/ml for UM and 100µg/ml with MM). Apparently the extracts UM showed good values compared to (MM) method it with two solvent.

Keywords: Calligonum comosum, Polyphenols, flavonoids, Antioxidants activities, Ultra-sound (UM), Maceration (MM).

Introduction
Calligonum comosum L'her. (Polygonaceae)., plant has many local names such as Ghardaq in Arabian Peninsula or Larta and Ouarach in Algeria [1]. It is a small shrub leafless, distributed throughout Arabia and growing in sandy deserts [2]. It is used by The Bedouins to treat stomach ailments illnesses [3]. In folklore medicine, *Calligonum* has a great reputation as a stimulant and astringent [4], the stems are chewed for curing toothache [1]. The Dehydrodicatechin isolated from this plant by [5] showed best antioxidant and cytotoxic activities. As well the anthraquinones of *Calligonum comosum* manifested high antimicrobial potential [6]; the efficacity of ethanolic extract of *Calligonum comosum* against *fascioliasis* animal disease was confirmed by [7].
The aims of this study were to choice the solvent and the extraction method that can used to obtain the best yield on phenolic contents (Total Polyphenols and flavonoid) and antioxidant activities from *Calligonum comosum* growing in Oued Souf Saharan region (south-east of Algeria).

2. Materials and methods
2.1. Biological material:
Calligonum comosum was harvested in October 2014 during the somatic stage in Oued Souf region (South-east of Algerian Sahara). The plant dried, crushed and stored in glass flasks to protect from light and moisture for subsequent analysis.

2.2. Preparation of extracts:
In this study, two extraction methods were used; with methanol and ethanol solvents in both methods:

2.2.1. Maceration:
5g of dried drogue was introduced in 50ml of organic solvent for 24h. After filtration, this solution was evaporated by rotary evaporator Type Buchi R-200 at 55°C for methanol and 60°C for ethanol maceration [8].
2.2.2. Extraction with ultra-sound:
According to [9] with slight modification, 50ml solvents is added to 5g of dried drogue material then take mixtures to ultra-sound type JP SELECTA (3.1A; 720W) at 30°C for 30 min. The extracts were evaporated in rotary evaporator.
According to [10] the yield of the extracts was determined by the formula:

Yield (%) = (P_1 / P_2) x 100.

- P_1: Weight of the extract dried in gram.
- P_2: Weight of the plant starting material in gram.

2.3. Determination of Total Polyphenolic Contents:
The total polyphenolic content determined according to the method described by [11]. 0.2 ml of the extract was mixed with 1 ml of Folin-Ciocalteu reagent (diluted 10 times). Then 0.8 ml of solution of Na_2CO_3 (7.5%) was added to the mixture. The mixture was incubated at room temperature, protected from light, for about 30 minutes. The absorbance is measured at 760 nm. The results are expressed in mg equivalent Gallic acid/g DM.

2.4. Determination of Flavonoids
The flavonoids were estimated using method of [12]. 0.5 ml of extracts, 0.5 ml of $AlCl_3$ (2%). After 60 min at room temperature, the absorbance was measured at 420 nm. A yellow color indicates the presence of flavonoids. Samples of extracts ware evaluated at 0.1 mg/ml. Total flavonoid contents was calculated as Quercetin (mg equivalent /g DM).

2.5. Antioxidants tests:
In this study the identification of antioxidant activity *in-vitro* extracts of *Calligonum comosum* was performed by two tests respectively: the free radical scavenging DPPH and Total Antioxidant Capacity TAC (test phosphor molybdenum).

2.5.1. Scavenging free radical DPPH test:
The antioxidant activity of different extracts was measured by the method described by [13] with a slight modification: 0.5ml of methanol and ethanol extracts of *C. comosum* with 1ml DPPH (0.1mM), for positive comparison we used two standards antioxidant: Ascorbic acid form ethanol extracts and α-Tocopherolfor the ethanol extracts. The tubes were incubated at 37°C for 30 min. The absorbance was measured at 515 nm. Percent inhibition is determined by the following formula:

$$\%\text{DPPH radical scavenging} = [(A_c - A_s)/A_c] \times 100.$$

Where A_c is the absorbance of the control and A_s is the absorbance of the sample.

2.5.2. Determination of total antioxidant capacity by the phosphor molybdenum method:
The antioxidant activity of different *C. comosum* extracts were evaluated by the phosphor molybdenum method of [14]. 0.1ml of extracts solution was combined with 1ml of reagent solution (0.6M sulfuric acid, 28mM sodium phosphate, and4mM ammonium molybdate).The tubes were capped and incubated in water bath at 95°C for 90 min. After the samples were cooled, the absorbance of each was measured at 695nm. The antioxidant capacity was expressed as an equivalent of Gallic acid (mg Gallic acid /g dried extract).

2.5.3. The IC_{50} Determination:
The effective concentration having 50% radical inhibition activity (IC_{50}), expressed as µg extract/ml, was determined from the graph of the free radical scavenging activity (%) against the extract concentration [15].

3. Results and discussion

3.1. Yield of extracts:
The yield of the ethanol extracts (fig.01) was low (6.99% in ultra-sound and 4.61% in maceration) compared to the methanol extracts (10.91% in ultra-sound and 10.78% in maceration). The results obtained in this study are different comparing with others data, because the solvent used and preparing conditions are not similar [16].

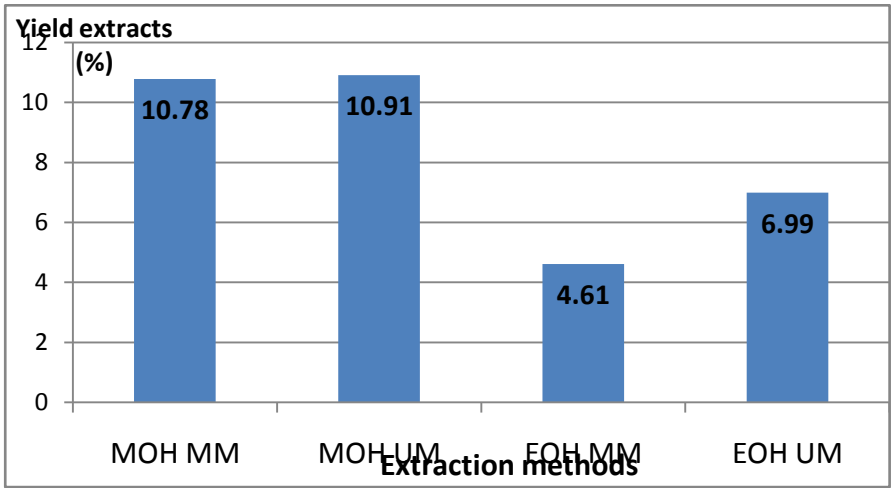

Figure 1: Yield extracts of *C. comosum* (MOH MM: methanol extract by maceration; MOH UM: methanol extract by ultra-sounds method; EOH MM: ethanol extract by maceration method; EOH UM: ethanol extract by ultra-sounds method).

3.2. Polyphenols and flavonoid Contents:

The polyphenol quantity and flavonoid was determined from calibration lines (regression line) using the standard Gallic acid and Quercetin in ethanol and methanol.

The concentration is expressed in milligrams of Gallic acid equivalents per gram of dry materiel (mg GAE / g DM) by polyphenol and in milligrams Quercetin equivalents per gram of dry materiel (mg QE / g DM) by flavonoid Table (1). The results showed that the extracts of *C. comosum* contained Phenolic compounds in the following order: EOH MM>EOH UM>MOH MM>MOH UM.

The variability observed in the values of polyphenols quantity through the different extracts of *C. comosum* could be attributed to the difference in the phenolic composition of the samples studied [17].

Table 1: Effect of extractions methods and solvent in phenolics contents of *C. comosum*.

Extracts of *C. comosum*	MOH MM	MOH UM	EOH MM	EOH UM
Total polyphenols (mg GAE/g DM)	3,28 ± 0,25	1,55 ± 0,32	175,75 ± 6,4	173,24 ± 5,7
Flavonoids (mg QE/g DM)	1,19 ± 0,14	0,51 ± 0,14	38,59 ± 1,89	32,97 ± 0,78

3.3. Antioxidant activities:

3.3.1. DPPH Test:

The highest scavenging activity of DPPH radical in concentration 0.01mg/ml (Figure 2) was found for the EOH UM extract (69.42%), followed by the EOH MM extract 60%. The Ascorbic acid, MOH MM and MOH UM extracts exhibited the lowest scavenging activities with values of 37.40; 29,67 and 19,34% respectively. It also gave the α-Tocopherol in low concentration (0.0001 mg/ml) best rate of inhibition with DPPH radical 40.16%.

3.3.2. Molybdate ion reduction assay (Antioxidant Capacity Total):

The results indicated that the EOH MM extract of *C. comosum* had the highest antioxidant capacity with a value of 596.83 mg Gallic acid equivalent/g dried extract. The second place occupied by the EOH UM extract with a value of 511.22 mg Gallic acid equivalents/g dried extract. The methanol preparations (MOH MM and MOH UM) showed lower antioxidant capacities with values ranged between 275.41 and 231.59 mg Gallic acid equivalents/g dried extract (Figure3).

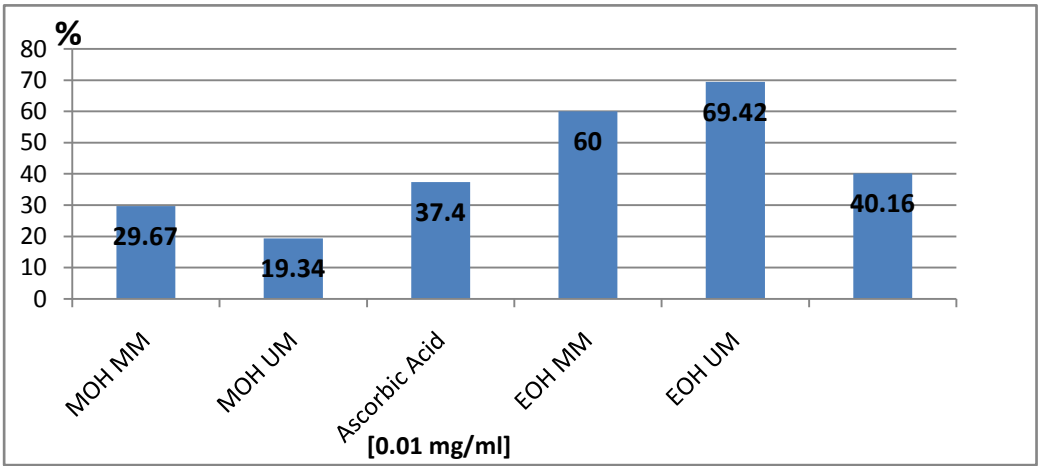

Figure 2: Higher of rate inhibition (%) of free radical DPPH.

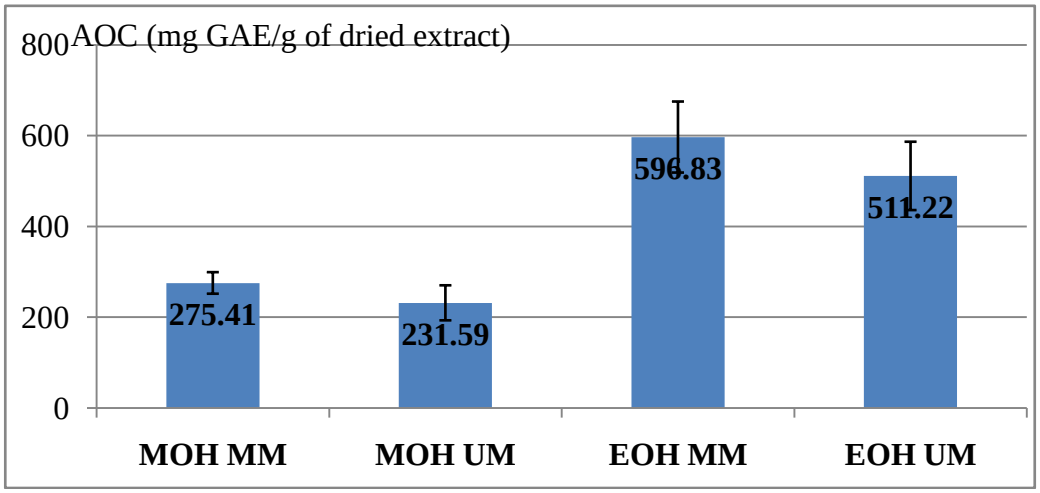

Figure 3: The variation of antioxidant capacity for different extracts of *C.comosum*.
AOC: Anti-Oxidant Capacity; GAE: Gallic acid equivalent.

3.3.3. The IC_{50} of DPPH Test:

The values of the antioxidants (IC_{50}) effects of different extractions methods reveals (Figure 4) that the lower value obtained with α-Tocopherol (2.1 µg/ml) following by ethanol extract method respectively EOH UM(6.3 µg/ml)< EOH MM (7.9 µg/ml). But the methanol extracts gives the highest values (MOH UM: 50µg/ml, MOH MM: 100µg/ml).

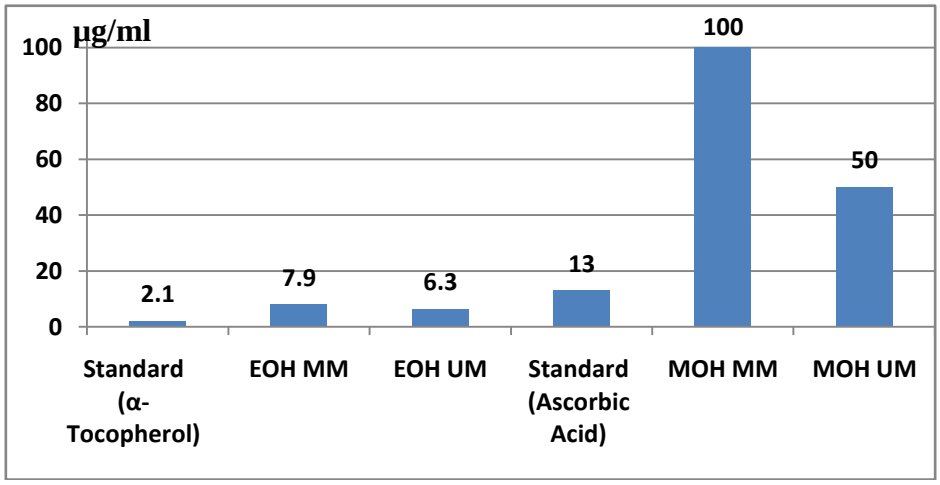

Figure 4: IC_{50} values (µg/ml) of extracts of against free radical (DPPH).

It was clearly noticed superiority ethanol extracts to methanol extracts in IC_{50} values. This was due to the effectiveness of anti-oxidation which was closely linked to the structure and quality of phenolic compounds than the concentration and quantity of these compounds within the plant tissue [18].
The strong effect of anti-oxidants in some samples of ethanol extracts could be explained by the difference in antioxidant activity between samples for different behaviour to give a proton and an electron between samples [19].

Conclusion

The evaluation of effects of solvents and extraction methods on the phenolic contents and antioxidant activities for the medicinal plant *Calligonum comosum* L'her., growing in Oued Souf Saharan region (south-east of Algeria), revealed that the optimum yield of extracts obtained with methanolic extracts especially with the ultra-sound method, but significantly overtook the etanolic extracts in the amount of polyphenols. Also, it was not recorded any significant differences between the two methods of extraction by using different solvent. As the antioxidant activities, we noticed that ethanol was the best organic solvent used for extracting the major parts of phenolic contents. These results were explained by scavenging activity of DPPH radicals. The total antioxidants capacity indicated that the ethanolic extract has the highest antioxidant capacity.
The IC_{50} values revealed that the ethanolic extracts were important compared with methanolic one. Also the Ultra sound method give the higher values of IC_{50} compared with macerations methods in both solvents.

References

1. Chouikh A., Mekki M., Adjal E. H., *Int. J. Recent. Sci. Res.* 6(4) (2015) 3534-3536.
2. Halis Y., *Plant Encyclopedia in area Oued Souf: desert plants common in the Big East race*, Edition El Walid, El Oued, Algeria. (2007) 62-63.
3. Liu X. M., Zakaria M. N. M., Islam M. W., Radhakrishnan R., Ismail A., Chen H. B., Chan K., Al-Attas A., *Fitoterapia*. 72 (2001) 487-491.
4. Muschler R.,. *A Manual Flora of Egypt*. R. Friedlaender & Sohn Edition, Berlin, Germany. (1912).
5. Badria F. A., Ameen M., Akl. R. M., *Z. Naturforsch.* 62c (2007) 656-660.
6. Zaki D., Abd-El-Aziz M., El-Gengeihy S., Morsi N., *Herba. Hung*. 23 (1984) 73-84.
7. Degheidy N. S., Sharaf E. M., Fathi S. M., *Global. Veterinaria*. 11(4)(2013) 377-384.
8. Rebaya A., Belghith S. I., Baghdikian B., Leddet V. M., Mabrouki F., Olivier E., Cherif J. k., Ayadi M. T., *J. Appl. Pharm. Sci.* 5 (1) (2015) 52-57.
9. Khosravi M., Mortazavi S. A., Karimi M., Sharayie P., Armin M. *Intl. J. Agri. Crop. Sci.* 5(21) (2013) 2521-2528.
10. Matkowski A., Piotrowska P., *Fitoterapia*. 77(5) (2006) 346-353.
11. Singleton V. L., Rossi J. A., *Am. J. Enol. Vitic.* 16 (3) (1965) 144-158.
12. Ordonez A.A.L., Gomez J.D., Vattuone M.A., Isla M.I., *Food. Chem*. 97 (2006) 452-458.
13. Sliti S., Ayadi S., Kachouri F., Khouja M. A., Abderrabba M., Bouzouita N., *J. Mater. Environ. Sci.* 6 (2) (2015) 531-537.
14. Yazdanparast A. R., *Food. Chem. Toxicol*. 45 (2007) 2402-2411.
15. Dziria S., Hassenb I., Fatnassia S., Mrabeta Y., Casabiancac H., Hanchid B., Hosnia K., *J. Funct. Foods.* 4 (2012) 423-432.
16. Najjaa H., Neffati M., Zouari S., Ammar E., *C. R. Chim.* 10 (2007) 820-826.
17. Hayouni E., Abedrabba M., Bouix M., Hamdi M., *Food. Chem*. 105(3) (2007) 1126-1134.
18. Rice-Evans C. A., Sampson J., Brameley P. M., Holloway D. E., *Free. Radical. Res.* 26 (4) (1997) 381-398.
19. Miliauskas G., Venskutonis P. R., Van Beek T. A., *Food. Chem*. 85 (2) (2004) 231-237.