

Phenolic and Flavonoid Contents of Some Plant Extracts from Tunisia Southern Landscape by Using Different Extraction Techniques: The Case of *Retama reatam*

Asma Rejab^{1,2}, Hatem Ksibi^{*1,3}

¹University of Sfax, LASEM-ENIS, PB BPW 3038, Sfax, Tunisia; ²Gabès University, ENIG Avenue Omar Ibn El Khattab, Zrig Gabes 6029, Tunisia; ³University of Sfax, IPEIS PB 1172 Sfax 3018 Tunisia

ABSTRACT

Nowadays traditional medicine, a source of several bioactive molecules for therapeutic purposes, has become a cure for various diseases. In fact, the evaluation of plant exploitation has become progressively significant and this for their therapeutic effects of many traditional medicines may be due to the immense presence of natural antioxidants. In this respect, *Retama reatam* was chosen among six herbal Tunisian plants traditionally known for their therapeutic virtues, as the best source of total polyphenol content (1122,927g GEA/g extract) with an important extraction yield as ethanol is the solvent. The Soxhlet extraction always has the lowest value in total polyphenol content (164,857 GEA/g extract) for such solvent. The total flavonoid content of the different extracts is not significantly different from each other. However, the yield extraction remains higher for ethanolic extraction exceeding a value of 26.7%. Concerning antioxidant activity of *Retama reatam*, results suggest that supercritical CO₂ extraction can be used as an efficient alternative for pre-treatment to eliminate fatty compounds and therefore evaluating oxygen radical absorbance capacity values.

Keywords: Medicinal plants; Extraction; *Retama reatam*; Total polyphenols content; Total flavonoid content; Antioxidant activity ORAC

INTRODUCTION

Traditionally, herbal materials are known as a prominent medicinal source of polyphenols particularly flavonoids and represent a class of bioactive molecules that are closely related to multiple health benefits. Saharian and southern Tunisian ancestors prepared folk medicaments to cure various diseases using several plants growing spontaneously, although there is little information about composition, contents and related effects.

The interest in plant and herbal materials has become gradually significant may be due to the presence of natural antioxidants such as phenolic plant compounds and flavonoids. In this respect, the development of a standard method for efficient and rapid extraction of these secondary metabolites remains a challenge.

Indeed, there is a diversity of species with therapeutic interests, but many of them are not studied. For example, very few books have been devoted to *Retama reatam* which is spontaneous shrub belonging to the Fabaceae family, locally known as R'tem. This virtue plays an important ecological role and is widely used in dune

stabilization and soil fixation [1]. In addition, it is usefully used for the treatment of several diseases such as diabetes, Conforti et al. [2], eczema and hypertension, Alfalluos et al. [3].

The aim of this study is to investigate a qualitative and quantitative phytochemical analysis and antioxidant activity for medicinal plants as *Ratema raetem*, *Artemisia alba*, *Elymus repens*, *Diplotaxis harra*, *Kochia scoparia*, *Lawsonia inermis*, especially. Firstly, this study will focus on determining the richest TPC source among tested plants using maceration as a conventional extraction technique. Secondly, to give data: extraction yield efficiency, TPC, TFC and antioxidant activity of the chosen herbal materiel (*Retama reatam*) following several extraction techniques.

MATERIALS AND METHODS

Plant sampling

All plants are traditional medicinal plants present in humid bioclimatic regions in arid zones of Tunisia and used for their therapeutic effect. Different leaves and seeds plants were handily

Correspondence to: Hatem Ksibi, University of Sfax, LASEM-ENIS, PB BPW 3038, Sfax, Tunisia, Tel: (+216) 98 906 120; E-mail: hatem.ksibi@ipeis.rnu.tn

Received: July 29, 2019, **Accepted:** September 09, 2019, **Published:** September 16, 2019

Citation: Rejab A, Ksibi H (2019) Phenolic and Flavonoid Contents of Some Plant Extracts from Tunisia Southern Landscape by Using Different Extraction Techniques: The Case of *Retama reatam*. *Med Aromat Plants* (Los Angeles) 8:337. doi: 10.35248/2167-0412.19.8.337

Copyright: © 2019 Rejab A, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

gathered in Tunisia southern landscape on spring season between March and May in 2018. The identity of plants is confirmed at Laboratory of Bio-resources: Integrative Biology and Valorization (LR14-ES06), University of Monastir (Tunisia). The harvested products are well cleaned and rinsed with distilled water then dried in shade at room temperature ($\sim 20^{\circ}\text{C}$) for 3 weeks. Such drying process may avoid undesirable thermal degradation of the finished products until reaching a new stable form and a constant weight. Materials are chopped and ground separately to fine powders in a mechanical blender. The obtained powders are well-kept in hygienic plastic containers in a dark room to avoid effect of light, heat and moisture until use.

Our interest is focused on metabolic properties of 6 types of plants belonging to the Tunisia southern landscape. They are considered as those occurring spontaneously in the Tunisian Sahara and steppes. Hence, we propose a comparative study of various extraction methods applied to leaves of *Retama raetam*, *Artemisia alba*, *Elymus repens*, *Diploaxis harra*, *Kochia scoparia*, *Lawsonia inermis* (Figure 1). These spontaneous plants are usefully proved in local folk medicine and studied by several previous works [4-6].

Chemicals

All chemicals are of analytical reagent grade chemicals. Carburios Metálicos S.A. (Spain) supplied carbon dioxide (mass fraction purity 0.999 in the liquid phase). Folin-Ciocalteu reagent, gallic acid, catechin, are gotten from Sigma Aldrich (Spain). Other reagents are of analytical grade or higher available purity.

Extraction procedures

There are several methods for extracting polyphenols and for selecting one plant among others, maceration with ethanol and hexane is used. Two types of sampling are prepared for each plant (two solvents of different polarity): hexane (apolar) and ethanol (polar). We also use Solid liquid, Soxhlet and Supercritical fluid extraction techniques to extract *Retama raetam* and evaluate TPC, TFC and antioxidant activity.

Solvent choice: Two solvent systems are selected: ethanol as polar solvent (96%) and hexane as apolar solvent (99%) where solubility of such phenolic compound is strongly related to the solvent polarity. Indeed, the nature of extraction solvent can have a significant impact on the extraction yield of polyphenols from plant material [7].

Maceration: It is the simplest mode of extraction where the powdered plant material is taken inside an airtight container and soaked with the solvent for a specified period of time until the soluble portions are dissolved in the solvent (60, 90, and 180 minutes). A series of experiments aims at varying the extraction parameters (temperature, extraction time and solvent volume) are evaluated in order to optimize the TPC as well as the TFC. The operation is repeated several times to identify the best parameters. The resulting mixture is filtered with filter paper and put in a box knowing that the moisture of *Retama* is 13.60%. 10 g of each plant are weighed and placed in the filter cartridge, then in a box in which 200 mL of solvent (96% ethanol then hexane) is added. This extraction step is stopped after 6 days when the liquid surrounding the cartridge takes the plant color. This color indicates that the solvent no longer extracts anything from the solid. The various extracts obtained are filtered on Whatman paper No. 4 (Ashless filter paper) and stored at 4°C . for subsequent analyzes.

Solid liquid (SL) extraction: The conventional SL extraction is carried out by batch ethanolic extraction from *Retama raetam* in a stirred vessel. Several experiments for the investigated system are carried out in order to obtain the optimum conditions for extraction. The formed solution is stirred for eliminating external mass transfer resistance. Experiments are performed at both 40°C and 60°C . Each extraction experiment lasts 90 minutes in order to reach equilibrium state of the process.

Soxhlet extraction: Crushed *Retama raetam* leaf powder (10 g) is packed into a Soxhlet apparatus and extracted with 100 mL hexane at 40°C for 6 hours. The extract is filtered through Whatman filter paper No. 1, and the filtrate is concentrated under evaporation to dryness at 100°C using an oven. The extract is dried, weighed (8.98



Figure 1: Plant varieties (1) *Retama raetam* (2) *Artemisia-alba* (3) *Elymus repens* (4) *Diploaxis harra* (5) *Kochia scoparia* (6) *Lawsonia inermis*.

g) and stored at 4°C in storage vials till their usage in subsequent experiments. Experiments are achieved in duplicate.

SFE extraction: Supercritical Fluid Extraction (SFE) is considered an environmentally friendly and a novel extraction technique that allows for selective extraction using a supercritical solvent. Supercritical fluid extraction technology has advanced tremendously since its inception and currently represents the method of choice in many food processing industries [8]. The extraction tank is loaded with 90 g dried *Retama reteam* leaves. The system is used at a constant temperature of 45°C and a pressure of 280 bars to eliminate the waxy cuticles and other fatty compounds from the ethanolic extraction. For the experiment, conditions in the recovery section are defined at 46 bars/15°C in the separator. The CO₂ flow rate is 2 kg/h and the extraction is complete after 75 min. The obtained mass from the extractor weighs 87.5 g. Then this extract is subjected to a series of solid liquid extractions with ethanol to get a final mass from separator close to 7.26 g.

Characterization

Total phenolic content: Total phenolic content (TPC) is determined by the Folin-Ciocalteu method and expressed as milligram gallic acid equivalents per gram of dry product (mgGAE/gDB) (Waterhouse 2002). An aliquot of 40 µL of the extract is diluted in 3 mL of milliQ water and allowed to react during 5 min with 200 µL of Folin-Ciocalteu reagent. After this, 600 µL of sodium carbonate (20%, saturated) is added. After, the mixture is left for 30 min at 40°C. Absorbance of each sample is measured at 765 nm against the blank in a UV 2550 Shimadzu spectrophotometer and compared against a gallic acid calibration curve (112–900 ppm). For quantification of the total phenolic in the extract, gallic acid is used as the reference compound so Total phenolic content of the extract samples is expressed as Gallic Acid Equivalent (GAE) milligrams per gram of the extract. All determinations are carried out in triplicate.

Total flavonoid content: Flavonoids and phenolic compounds in general are commonly known as plant secondary metabolites that hold an aromatic ring bearing at least one hydroxyl groups. The aluminum chloride colorimetric method is used for the determination of the total flavonoid content of the sample. Total flavonoid content (TFC) is characterized by a modification of the Yang procedure proposed by Pekal et al. (Pękal and Pyszynska 2014). An aliquot of 100 µL of the extract is poured into 1 mL of distilled water and mixed with 300 µL of NaNO₂ (5% w/v). After 5 min, 500 µL of AlCl₃ (2% w/v) is added. The solution has reacted for 6 min followed by neutralization with 500 µL of 1 M NaOH. The solution is stirred vigorously and further diluted by adding 10 mL of milliQ water. After 10 min, the absorbance is measured against a blank at 510-nm UV 2550 Shimadzu spectrophotometer. Measurements are quantified using a calibration curve made with catechin (60–500 ppm), and results are expressed as milligrams of catechin equivalents per gram of dry product (mgCAT/gDB). All samples are analyzed in triplicate.

Antioxidant activity: The oxygen radical absorbance capacity

(ORAC) assay is used as a standard tool to measure the antioxidant activity of polyphenols in extracted *Retama reteam* seeds via different methods. The antioxidant activity of polyphenols is due to their ability to scavenge free radicals, donate hydrogen atoms or electron, or chelate metal cations. The fundamental of these assays relies on the fluorescence quenching of disodium fluorescein (FS) salt after exposure to AAPH (2,2-azobis(2-amidino-propane) dihydrochloride), which generates oxygen radicals (ROO•) at a constant rate [9]. ORAC assay is carried out following the method developed by Huang et al. [10].

A 96 well-plate is used in a Fluostar Optima (BMG-Labtech) to determine the signal quench from fluorescent probe (100 nM Fluorescein in PBS) due to the reactive oxygen species, generated from the thermal decomposition of 240 mM AAPH [2,20-azobis(2 methylpropionamidine) dihydrochloride] at 37°C. Trolox (12.5–200 microM) standard (3-fold), or diluted extract samples or dissolution of the SAS powder (6-fold) are added in the wells. Depending on the antioxidant activity, the stability of the fluorescence signal is affected.

Fluorescence is recorded over time, and from integrating the area under the kinetic curves for each well, the antioxidant capacity is estimated (Optima-MARS Data Analysis). ORAC values of the extracts are compared to Trolox standards and expressed as Trolox Equivalents (TE) [micromol Trolox/g dry material].

RESULTS AND DISCUSSION

Yield

Extraction of the phenolic compounds using maceration is carried out with two types of solvents of different polarity: 96% ethanol as a polar solvent and hexane as a non-polar solvent. The extraction yield (mass of extract/mass of dry matter) is used as an indicator of the effects of the maceration conditions. In reflux, ethanol extract yield (15.36%) is maximum for *Retama reteam*. For *Lawsonia inermis* also, the trend is similar as observed in the case of refluxing. Whereas the extract yield is relatively lower than that with hexane (6.95%).

Figure 2 shows the comparison between yields of two solvents per plant. Generally, the yield is higher in ethanolic extracts than those treated with hexane. More precisely, the best yield is obtained for the ethanolic extract of *Retama reteam* with 15.36%; therefore, ethanol is about six times more effective than hexane in extracting phenolic compounds from *Retama reteam*. Similarly, for the other plants, *Artemisia* is twice, *kochia* almost three times, while for the *Elymus repens*, the difference between the two solvents is practically negligible.

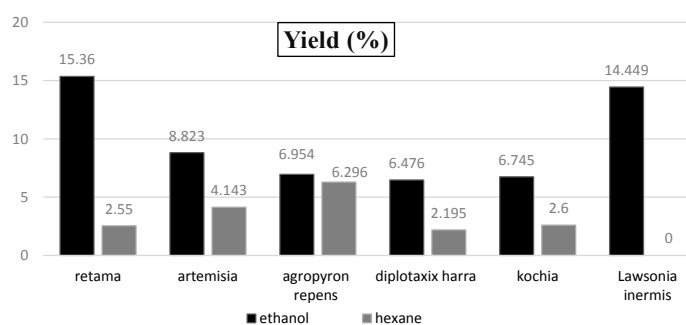


Figure 2: Maceration yields of extracts from several plants using both ethanol and hexane as solvents.

Experimentally, ethanol as a polar solvent is recommended and frequently used for the extraction of the phenolic compounds therefore several studies indicated that the phenols are moderate polar compounds [11]. In the case of hexane, the best yield is obtained with *Elymus repens* (6.296%), followed by *Artemisia alba* (4.143%). The other plants have almost the same yield, with a slight difference of 2.4% on average.

Total phenolic content

Results show that *Retama reatam* extract has the highest yield and the most important TPC compared to the other extracts. Comparing the total polyphenol content value by maceration technique lasted after 6 days, 1123-mgGAE/g dry extract, TPC for other extracts following different extractions is lower, this may be due to the extraction time at a given temperature and influenced by ethanol concentration [12]. In fact, a longer extraction time can lead to increase exposure to temperature, light and therefore to increase TPC values relatively to different bioactive compound with diverse structures.

A similar trend is observed for TPC when maceration technique results are compared to SFE extraction ones while Soxhlet extraction still has the lowest TPC value. Indeed, *Retama reatam* chromatograms from different saharian regions were published in several papers such as Hammouche-Mokrane et al. and Awen et al. [13,14]. These data allowed us to valid the richness of *Retama reatam* with phenolic compounds.

Similarly, the TFC of the other extracts do not show significant difference from each other, the value varies between 5.366 g EC/g. extract for the SFE extraction and 6.0474 for the ethanolic extraction. On the other hand, the yield is however higher for stirred maceration with a value of 26.7%.

Based on the comparison of the different extraction methods used for polyphenols, the best results are shown for the extracts obtained through the conventional extraction method (ethanolic) indicating a higher selectivity for the extraction of antioxidants compounds.

The advantage of this technique is that it is a high efficiency extraction and can be done easily. In addition, easy maintenance of extraction vessels and improved recovery and repeatability are also better with this conventional technique than with new extraction techniques.

Total flavonoid content

As reported in histogram plots (Figure 3), we notice that *Retama reatam* TPC values exceeds 20 mg GAE/g for all used extraction techniques except soxhlet method. The obtained data are also validated through comparison with experimental results (ref 1 in histograms, (Figure 4) of Saada et al. In fact, the solvent extraction is shown as a useful technique for dealing with relatively plant samples. Identically, The TFC data are also presented solid-liquid extraction (40°C and 60°C), soxhlet and supercritical fluid extraction. The TFC in *Retama reatam* leaves varied from 5.04 to 5.76(mg EC/g. extract). Differently to TPC, we note that *Retama reatam* TFC varies slightly with the temperature and also according to different extraction techniques.

Concerning TPC and TFC measurements, the *Retama reatam* leaves extracts are obtained from various extraction protocols. Data given in Figures 4a and 4b represents mean standard deviation. *Retama reatam* extracts TPC TFC were measured as previously described. In brief, We assign SL40 and SL 60 the solid-liquid extraction done at 40°C and 60°C respectively, SFE the supercritical fluid extraction; and ref indicates published data of Saada et al.

Determination of antioxidant activity

The ORAC assay is able to differentiate the suitable *Retama reatam* extraction method which had a superior antioxidant activity. According to Figure 5 SFE (supercritical fluid extraction) has the highest ORAC value when is followed by SL (solid liquid extraction) and maceration, and soxhlet had mid-range ORAC values.

Furthermore, the finding showed that, there is no correlation between ORAC and TPC *Retama* extracts at a given extraction method. The lowest *Retama* TPC is seen when *Retama reatam* is extracted with SFE technique cannot reflect the high value of ORAC of *Retama* extracted with the same technique.

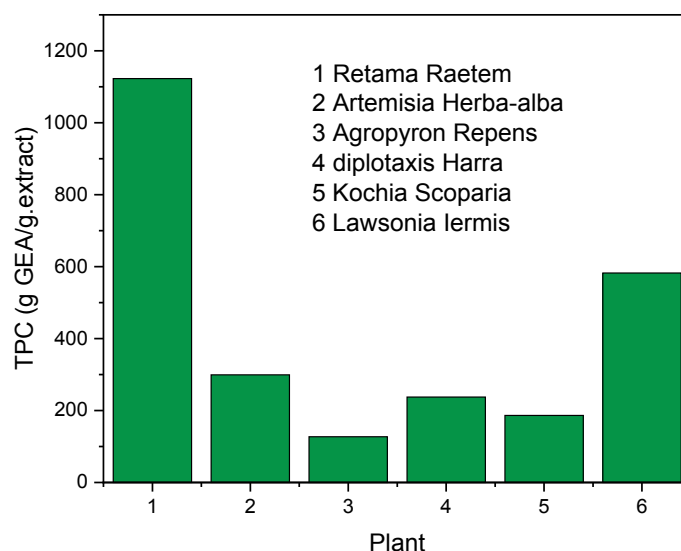


Figure 3: Histogram of total phenolic content of selected vegetables extracted with ethanol.

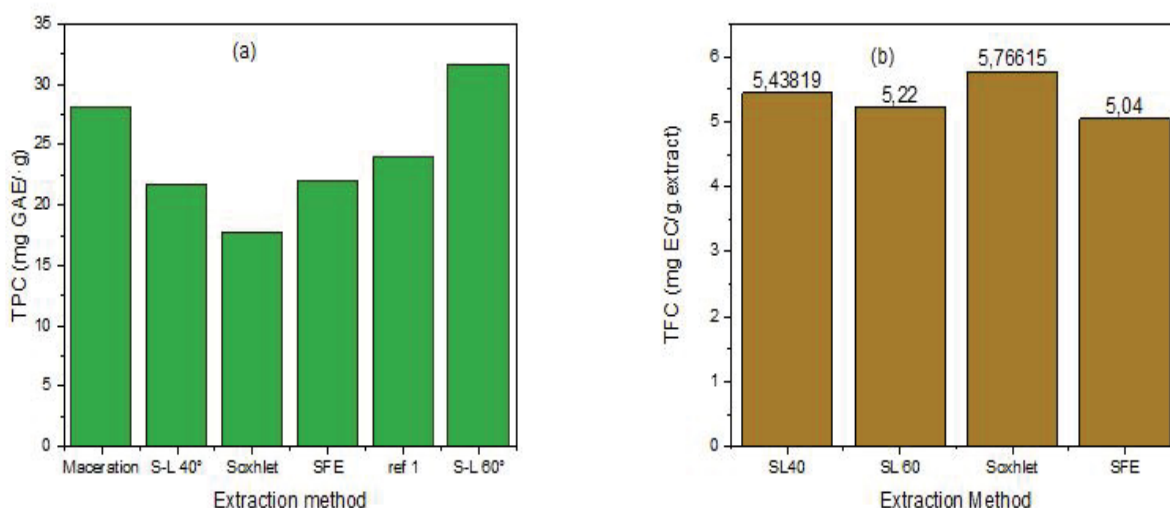


Figure 4: Different levels of TPC and TFC of *Retama reatam* extracted following several manners.

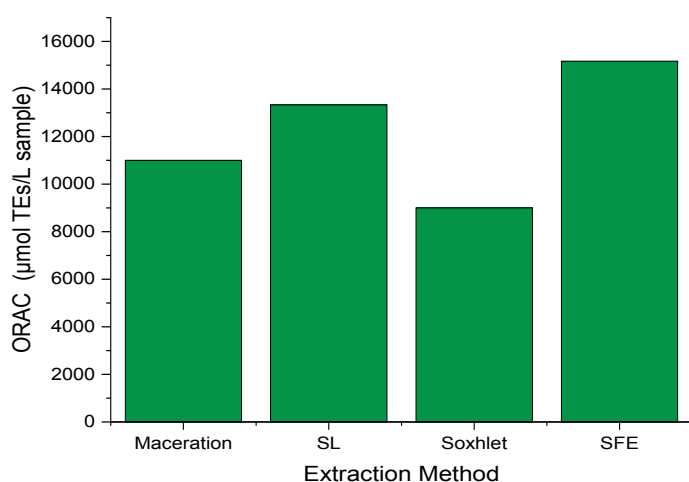


Figure 5: ORAC-FL values obtained by different extraction methods in *Retama reatam*.

CONCLUSION

In this study *Retama reatam* extracts obtained through ethanolic extraction and supercritical extraction, in three samples, indicates a higher TPC and ORAC for the extraction of herbal material from Tunisia southern landscape. The effect of using various extraction methods on polyphenols, flavonoids, and antioxidant activity of *Retama reatam* plant is shown. The results indicate that TFC is slightly higher in ethanol maceration, whereas between SFE and soxhlet there is no statistically significant difference. Depending on the polyphenol content, the highest grade is for the first maceration (6 days). This study allowed the optimization of a simple, fast and precise method for the determination of the TPC TFC and ORAC in leaves of *Retama reatam*, which can be used to support the quality assessment of this Tunisia southern herbal material.

ACKNOWLEDGMENTS

Authors gratefully thank the “Departamento de Ingeniería Química y Tecnología del Medio Ambiente” at The University of Valladolid (Spain), for giving the opportunity of a training period during which Asma Rejab has been developed the experimental work.

REFERENCES

1. Ferchichi A. La lutte contre l'ensablement et pour la stabilisation des dunes: Essai de la fixation biologique des dunes en Tunisie presaharienne. *Recherches sur la desertification dans la Jeffara Rev Tunis Geogr* 1996;12:49-102.
2. Conforti F, Statti G, Tundis R, Loizzo MR, Bonesi M, Menichini F, et al. Antioxidant and cytotoxic activities of *Retama reatam* subsp Gussonei. *Phytotherapy Research*. 2004;18:585-87.
3. Alfalluos KA, Alnade H, Wesam S, Kollab A, Alafid F, Edrah SM, et al. Qualitative and quantitative phytochemical analysis and antimicrobial activity of “Retama” extract grown in Zliten Libya. *Inter J of Med Sci and Clin Inv*. 2017;4:2861-66.
4. Uma DB, Ho CW, Wan Aida WM. Optimization of extraction parameters of total phenolic compounds from henna (*Lawsonia inermis*) leaves. *Sains Malaysiana*. 2010;39:119-128.
5. Saada M, Falleh H, Jalleli I, Ksouri MR. Phenolic profile, biological activities and fraction analysis of the medicinal halophyte *Retama reatam*. *South African Journal of Botany*. 2014;94:114-21.
6. Bahloul N, Bellili S, Aazza S, Chérif A, Faleiro ML, Antunes MD, et al. Aqueous extracts from Tunisian diplotaxis: Phenol content, antioxidant and anti-acetylcholinesterase activities, and impact of exposure to simulated gastrointestinal fluids. *Antioxidants (Basel)*. 2016;2:5-2.
7. Martinez-Correa HA, Bitencourt RG, Kayano AC, Magalhães PM, Costa FT, Cabral FA. Integrated extraction process to obtain bioactive extracts of *Artemisia annua* L. leaves using supercritical CO₂, ethanol and water. *Ind Crops and Products*. 2017;95:535-42.
8. Stalikas CD. Extraction, separation, and detection methods for phenolic acids and flavonoids. *J Sep Sci*. 2007;30:3268-95.
9. Garrett AR, Weigel EG, Martinez AD, Heaton M, Robison RA, O'Neill KL. Novel method for predicting antioxidant activity based on amino acid structure *Food Chem*. 2014;158:490-496.
10. Huang D, Ou B, Hampsch-Woodill M, Flanagan JA, Deemer EK. Development and validation of oxygen radical absorbance capacity assay for lipophilic antioxidants using randomly methylated β-cyclodextrin as the solubility enhancer. *J Agric Food Chem*. 2002;50:1815-21.
11. Wei SD, Zhou HC, Lin YM. Antioxidant activities of extract and fractions from the hypocotyls of the mangrove plant *Kandelia candel*. *Int J Mol Sci*. 2010;11:4080-4093.
12. Chew KK, Khoo MZ, Ng SY, Thoo YY, Aida WW, Ho CW. Effect of ethanol concentration, extraction time and extraction temperature

- on the recovery of phenolic compounds and antioxidant capacity of *Centella asiatica* extracts. International Food Research Journal. 2011;18:571-78.
13. Hammouche-Mokrane N, León-González JB, Navarro I, Boulila F, Benallaoua S, Martín-Cordero C. Phytochemical profile and antibacterial activity of *Retama raetam* and *R. sphaerocarpa* cladodes from Algeria. Natural Product Communications. 2017;12:1857-60.
 14. Awen BZ, Unnithan CR, Ravi S, Kermagy A, Sasikumar JM, Khrbash AS, et al. Essential oils of *Retama raetam* from Libya: Chemical composition and anti-microbial activity. Nat Prod Res. 2011;25:927-33.