

## Antioxidant activity and biochemical composition of fresh bulbs and leaves of wild garlic *Allium ursinum*

## Activité antioxydante et composition biochimique des bulbes frais et des feuilles fraîches de l'ail sauvage *Allium ursinum*

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**Abstract** – *Allium ursinum* commonly called wild garlic or bear's garlic is a medicinal species commonly recommended in traditional medicine for its therapeutic properties as well as common garlic. To the best of our knowledge, phytochemical investigations and antioxidant capacities of this plant remain unclear. This work assessed the phenolic composition of fresh bulbs and leaves of *A. ursinum* and evaluated their antiradical capacity according to the extraction method. Extraction was realized by two methods: infusion and decoction. Phenolic composition was determined by quantitative analysis of total phenolics, flavonoids and condensed tannins. Antioxidant activity was evaluated through the use of synthetic radical DPPH. Results showed that extraction method and organ influence biochemical composition and antioxidant activity. It has been observed that leaves were significantly richer in total polyphenols, flavonoids and condensed tannins than bulbs. Decoction gave the richest extract of polyphenols and flavonoids. However, infusion gave the richest extract of condensed tannins. The higher percentage of DPPH inhibition (66.61%) belong to the extract of fresh bulbs obtained by infusion. Because of the odor of the bulbs extract, we thought to analyze extracts with Gas Chromatography coupled to Mass Spectrometry (GC/MS). Our data showed that volatile composition of extracts is variable depending on the extraction method. Volatile fraction obtained by infusion was mainly composed of disulfide methyl propyl (46.45%) and disulfide dipropyl (53.55%). While decoction gave three compounds which are 1-limonene (41.74%), delta.-cyclogeraniolene (36.70%) and 3-butyl-1-cyclohexane (21.56%). Our findings showed that both organ and extraction method significantly affected biochemical composition and antioxidant activity.

**Keywords:** *Allium*, polyphenols, GC-MS, antioxidant activity, DPPH, infusion, decoction

**Résumé** -*Allium ursinum*, appelé ail sauvage ou ail des ours, est une espèce médicinale recommandée en médecine traditionnelle pour ses propriétés thérapeutiques comme l'ail commun. Au meilleur de nos connaissances, les investigations phytochimiques et les capacités antioxydantes de cette plante restent non claires. Ce travail s'intéresse à la composition phénolique des bulbes frais et des feuilles fraîches d'*Allium ursinum* et évalue leurs capacités anti radicalaire en fonction de la méthode d'extraction. L'extraction a été réalisée par deux méthodes: l'infusion et la décoction. La composition phénolique a été déterminée par l'analyse quantitative des polyphénols totaux, flavonoïdes et tannins condensés. Les résultats obtenus montrent que la méthode d'extraction et l'organe influencent la composition biochimique et l'activité antioxydante. Il a été observé que les feuilles sont significativement plus riches en polyphénols totaux, flavonoïdes et tannins condensés que les bulbes. La décoction donne l'extrait le plus riche en polyphénols et flavonoïdes. Tandis que, l'infusion donne l'extrait le plus riche en tannins condensés. Le pourcentage de l'inhibition de DPPH le plus élevé (66.1 %) appartient à l'extrait des bulbes frais obtenu par infusion. Grâce à l'odeur de l'extrait des bulbes, nous avons pensé à analyser les extraits par la Chromatographie Gazeuse couplée à la Spectrométrie de Masse (GC/MS). Nos données montrent que la composition volatile des extraits est variable dépendant de la méthode d'extraction. La fraction volatile obtenue par infusion était principalement composée de disulfide méthyle propyle (46.45%) et disulfide dipropyle (53.55%). Par contre, la décoction donne trois composés qui sont: 1-

limonène (41.74%), delta.-cyclogeraniolène (36.70%) and 3-butyle-1-cyclohexane (21.56%). Nos résultats montrent que, l'organe et la méthode d'extraction affectent significativement la composition biochimique et l'activité antioxydante

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**Mots clés :** *Allium*, Polyphénols, GCMS, activité antioxydante, DPPH, infusion, décoction.

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## 1. Introduction

Nowadays in this era of rapid improvements in medical technology, herbal preparations, known as alternative or complementary medicine, gained a lot of popularity (Qidwai and al, 2013). The increased interest in their use encouraged more detailed studies on plant resources (Vasile Bagiu et al, 2012). Antioxidants play an important role in inhibiting and scavenging free radicals, thus providing protection to human against infections and degenerative diseases. Current research is now directed towards natural antioxidants originated from plants due to safe therapeutics (Sreelatha and Padma, 2009). The antioxidant effect of plant products could be mainly ascribed to phenolic compounds, such as flavonoids, phenolic acids, tannins, and phenolic diterpenes. Phenolics play an important role in human health owing to their anti-inflammatory, antiallergic, antimicrobial, anticarcinogenic, and antiviral activities (Aaby et al, 2004). Further, selection of suitable extraction process and optimization of various parameters are critical for upscaling purposes i.e. from bench scale to pilot plant level. Various extraction techniques most commonly used include conventional techniques such as maceration, percolation, infusion, decoction, hot continuous extraction etc. (Bettaieb Rebey et al, 2012; Jalleli et al, 2012). Nevertheless, extract yield as well as the bioactivities of the extract prepared using different extraction methods have been reported to vary in several studies and thus literature about the most effective methods to extract these compounds is abundant but to some extent contradictory (Hayouni et al, 2007). It should be noted that a single extraction compared to multiple extraction procedure is not sufficient in the extraction of polyphenols (Turkmen et al, 2006). Considering the structure of these compounds and their physicochemical properties, it would be impossible to propose a universal extraction protocol.

Plant species represent a vast source of phytochemicals of varied chemical structure; many of them have already been studied extensively for their potential biological activities such as Labiatae, Compositae, Umbelliferae, Asteraceae, Polygonaceae and Amaryllidaceae. The genus *Allium* contains an estimated 750 species (Mathew, 1996). Several of the species or varieties, e.g. garlic, elephant garlic, onions, shallots, spring onions, leeks, welsh onions and chives, are well-known edible crop plants (Phillips et al, 1998). Others, particularly ramsons and crow garlic, which are not usually cultivated but grow wild, tend to be used in a minor culinary role. *A. ursinum* is a monocotyledonous plant of the Alliaceae family commonly called wild garlic or bear's garlic is a medicinal plant largely recommended in culinary preparations and traditional medicine for its therapeutic properties as well as common garlic (*A. sativum*). However, studies on this plant remain rare (Błażewicz-Woźniak et al, 2011; Preuss et al, 2001).

A wide array of therapeutic effects of garlic such as hypolipidaemic, antiatherosclerotic, hypoglycaemic, anticoagulant, antihypertensive, antimicrobial, anticancer, antidote (for heavy metal poisoning), hepatoprotective and immunomodulation have been reported (Agarwal, 1996; Augusti, 1977). However, the mechanisms of protection in these conditions are not well understood. Oxidative stress, arising as a result of imbalance between free radical generation and key endogenous antioxidant defence in tissues, plays a key role in the initiation and progression of almost all these conditions (Stearé et al, 1995). Therefore, the focus of research to elucidate garlic's medicinal properties has been largely concentrated on this aspect during the past decade. In this study, we were interested in determining biochemical composition and antioxidant activity of wild garlic (*A. ursinum*) collected from Djerba. As mentioned previously, biochemical composition and biological activities could vary depending on the organ and the extraction method; thus, their effects were also investigated.

## 2. Materials and methodes

### 2.1. Plant material

Wild garlic *A. ursinum* collected from Djerba in southern Tunisia in spring 2015 (March-April). It was identified at the Center of Biotechnology of Borj-Cedria. Then, fresh bulbs and leaves were separated and used for the extraction of phenolic compounds.

## 2.2. Extraction

Fresh bulbs and leaves were manually crushed using a mortar. Extraction was then performed by water. Two traditional extraction methods have been used: water infusion (Hydro module 20; at 30 minutes) and water decoction (Hydro module 20; at 30 minutes) (Sapunjeva et al, 2012). Extracts were filtered using whatman paper and conserved at 4°C till further use.

## 2.3. Preliminary phytochemical analysis

Total phenolic content was measured using a colorimetric assay developed by Dewanto and al., (2002). 125 µl of each extract was added to 500 µl of distilled water and 125 µl of yellow Folin-Ciocalteu reagent. After vigorous agitation and a 3 minutes rest, 1250 µl of 7% sodium carbonate (Na<sub>2</sub> CO<sub>3</sub>) solution were added to the mixture to fix polyphenols. Finally, distilled water was added to obtain a final volume of 3 ml. After 90 minutes incubation at room temperature in dark, absorbance was measured at 760 nm. Gallic acid was used as a standard. The standard curve was linear between 0 and 100 µg/mL gallic acid. Results were expressed as milligrams of gallic acid equivalent (GAE) per gram of fresh plant (FP) (mg GAE/g FP). Three replicates were performed for each extract.

Total flavonoid content was determined according to the method of Zhishen and al., (1999). 250 µl of each extract was added to 75 µl of 5% NaNO<sub>2</sub>. After 6 minutes incubation at room temperature, 150 µl of 10% aluminum chloride (Al Cl<sub>3</sub>) were added to the mixture. Then, after 5 minutes incubation at room temperature, 500 µl of NaOH (1M) were added to the mixture. Finally, distilled water was added to get a final volume of 2.5 ml. Absorbance was measured with reference to a blank without extract at 510 nm. Results were expressed as milligrams of catechin equivalents (CE) per gram of fresh plant (mg CE/g FP).

Condensed tannins content were determined according to the method of Sun and al. (1998) as follow: 50 µl of each extract were added to 3 ml of 4% (w/v) vanillin dissolved in methanol and 1.5 ml of concentrated HCl. After 15 minutes incubation in dark at room temperature, absorbance was measured at 500 nm. Catechin was used as a standard and results were expressed as milligrams of catechin equivalents per gram of fresh plant (mg CE/g FP).

## 2.4. Volatile organic compounds analysis by Head/space-GC-MS

Volatile organic compounds were analyzed using a Head/space (TELEDYNE TEKEMAR HT3TM) coupled with an Agilent GC-MS system (GC with 7890A, mass detector 5975C with Triple-Axis, insert XL MSD).

A 30 ml Headspace vials were used for the analysis, which were incubated 30 minutes in headspace oven at 40°C then transferred in heated line at 85°C to avoid condensation of volatile organic compounds that were injected in the GC inlet during one minute with a static mode. A HP-5 ms (5% phenylmethylsiloxane) column was used (30 m by 250 µm by 0.25 µ) with 1.6 ml/min flow and Helium N60 (99.99%) as carrier gas. Each run was performed during 38.5 minutes. The temperature of the oven was programmed at 50°C for one minute, raised to 120°C with a rate of 3°C/min then raised to 150°C with a rate of 6°C/min and finally it finished at 200°C with a rate of 20°C/min. Injection was realized in 250°C with a splitless mode, the auxiliary temperature was 250°C and the mass spectrometer was operating in EI mode (70 eV), quadruple and source temperature are respectively fixed at 150°C and 250°C with a full scan mode from 20/z to 550m/z.

The obtained data were analyzed using the HPChem integration program referring to NIST02 mass spectra search library.

## 2.5. Antioxidant activity

Antioxidant activity was evaluated by the free radical scavenging assay. Anti radical activity was measured using synthetic radical DPPH (1,1-diphenyl-2-picrylhydrazyl) according to the method described by Bersuder et al. (1998).

All the samples were made at the same concentration (30 mg/ml). 1 ml of each extract was added to 2 ml of DPPH solution (0.02% DPPH in 99.5% Ethanol) (0.2mM). After vigorous agitation, the mixture had a rest of 30 minutes in the dark at room temperature. The reduction of DPPH radical was measured at 517 nm. A control was conducted in the same manner, except that distilled water was used instead of plant extract. In its radical form, DPPH has an absorption band at 517 nm which disappears upon

reduction by antiradical compounds. Lower absorbance of the reaction mixture indicated higher DPPH free radical-scavenging activity.

DPPH radical-scavenging activity was estimated by the percentage of inhibition using the following formula:

$$\% \text{ Inhibition} = [(A \text{ control} - A \text{ Extract}) / A \text{ control}] \times 100$$

Where A control: absorbance of the control without extract

A Extract: absorbance of the extract with DPPH

Comparison of anti radical activity of each extract allows determining the most active extract against DPPH. The highest inhibition percentage corresponds to the highest efficiency of the extract.

## 2.6. Statistical analysis

Statistical analyses were effectuated with Statistica 8 software. Analyses of variance were realized with ANOVA test and Duncan test for multiple comparisons and determination of significant level.

## 3. Results and discussion

### 3.1. Extraction method effect on the phenolic composition of *A. ursinum* fresh bulbs and leaves

Biologically active compounds usually occur in low concentration in plants. An extraction technique is that which is able to obtain extracts with high yield and with minimal changes to the functional properties of the extract required (Quispe Candori et al., 2008). Extraction yield is dependent on the solvent and method of extraction (Do et al., 2013). In fact, the release of different phenolic compounds varies with the extraction process variables such as, matrix particle, solvent and temperature. Thought-provoking, many previous studies has shown that hot water (70-80°C) could extract effectively the phenolic compounds than cold water (Rangsriwong et al., 2009). Extraction efficiency is affected by the chemical nature of phytochemicals, the extraction method used, sample particle size, the solvent used, as well as the particular plant organ used for the extraction (Stalikas, 2007).

In this context, total polyphenols, flavonoids and condensed tannins of *A. ursinum* bulbs and leaves extracts were investigated on samples extracted using two methods: infusion and decoction. Regardless to the extraction method and the specific organ, composition on phenolic compounds ranged from 0.38 to 5.68 mg GAE. Results are reported in Table 1.

**Table 1.** Variability of phenolic composition with extraction method and fresh organ of *A. ursinum*

	Bulbs		Leaves	
	<i>Infusion</i>	<i>Decoction</i>	<i>Infusion</i>	<i>Decoction</i>
<b>Polyphenols (mg GAE/g FP)</b>	0.38 <sup>f,g</sup>	1.38 <sup>e</sup>	3.07 <sup>h</sup>	5.68 <sup>g,f</sup>
<b>Flavonoids (mg CE/g FP)</b>	0.02 <sup>g</sup>	0.1 <sup>g</sup>	0.54 <sup>e</sup>	0.38 <sup>d</sup>
<b>Condensed tannins (mg CE/g FP)</b>	1.59 <sup>d,c</sup>	0.47 <sup>e,d</sup>	2.15 <sup>c</sup>	1.39 <sup>d,c</sup>

\* GAE : gallic acid equivalent; FP : Fresh Plant; CE: Catechin equivalent.

Means with different superscripts (c-h) within a line indicate significant difference (P<0.0x).

Fresh leaves extracts obtained by different extraction methods were significantly richer in polyphenols, flavonoids and condensed tannins than fresh bulbs extracts. In fact, total polyphenols of extract obtained by decoction of fresh leaves (5.68 mg GAE/g FP) and bulbs (1.38 mg GAE/g FP) are significantly higher than those obtained by infusion. Moreover, decoction of fresh leaves and bulbs gives the highest amount of flavonoids, respectively 0.83 mg CE/g FP and 0.1 mg CE/g FP. The difference is statistically significant for leaves extract but not statistically significant for bulbs extract. However, infusion gives significantly the highest content of condensed tannins in leaves (2.15 mg CE/g FP) and bulbs (1.59 mg CE/g FP). Our results concerning the effect of extraction method on the amount of phenolic compounds are in agreement with previous studies of Sapunjieva et al. (2012), Ouedraogo et al. (2015) and Gîtin et al. (2012) which have demonstrated that the extraction method significantly influences the phenolic compounds.

Concerning the effect of the organ on phenolic contents, our data showed that polyphenols, flavonoids and condensed tannins were higher in leaves whatever the method used for the extraction. Our findings have been confirmed by other studies, especially those of Sapunjieva et al. (2012) and Oszmiański et al. (2013). This variability was probably due to the different functions that organ provides within the plant (Djurdjevic et al, 2003).

### 3.2. Volatile composition

Volatile fraction obtained by infusion was mainly composed of disulfide methyl propyl (46.45%) and disulfide dipropyl (53.55%). While decoction gives three compounds which are 1-Limonene (41.74%), delta.-cyclogeraniolene (36.70%) and 3-butyl-1-cyclohexane (21.56%) (Table 2).

**Table 2.** Variability of organic composition with extraction method of fresh bulbs from *A. ursinum*

	Compounds	Percentage (%)
<i>Infusion</i>	Disulfide, methyl propyl	46.45
	Disulfide, dipropyl	53.55
<i>Decoction</i>	3-butyl-1-cyclohexene	21.56
	1-Limonene	41.74
	Delta.-Cyclogeraniolene	36.70

Fresh leaves extracts obtained by different extraction methods are significantly richer in polyphenols, flavonoids and condensed tannins than fresh bulbs extracts. In fact, total polyphenols of extract obtained by decoction of fresh leaves (5.68 mg GAE/g FP) and bulbs (1.38 mg GAE/g FP) were significantly higher than those obtained by infusion. Moreover, decoction of fresh leaves and bulbs had the highest amount of flavonoids with 0.83 mg CE/g FP and 0.1 mg CE/g FP, respectively. The difference was statistically significant for leaves extract but not statistically significant for bulbs extract. However, infusion significantly had the highest content of condensed tannins in leaves (2.15 mg CE/g FP) and bulbs (1.59 mg CE/g FP). Our results concerning the extraction method effect on the amount of phenolic compounds was in agreement with previous studies which have demonstrated that the extraction method significantly influenced the phenolic compounds (Sapunjieva et al, 2012; Ouedraogo et al, 2015; Gîtin et al, 2012).

Concerning the effect of the organ on phenolic contents, our data showed that polyphenols, flavonoids and condensed tannins are higher in leaves whatever the method used for the extraction. Our findings have been confirmed by other studies, especially those of Sapunjieva et al. (2012) and Oszmiański et al, (2013). This variability was probably due to the different functions that organ provides within the plant (Djurdjevic et al., 2003). This richness of the leaves could be explained by the fact that these organs are the site of photosynthesis, which induce the formation of reactive oxygen species hence the need for the biosynthesis of potent antioxidants capable of their neutralization (Milane, 2004).

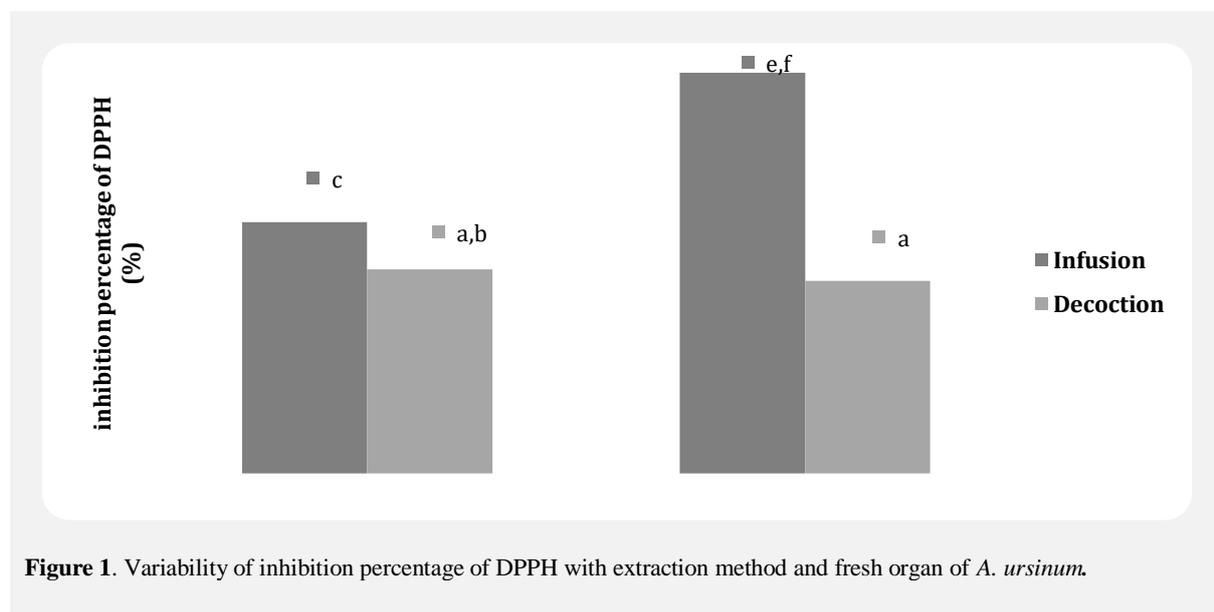
### 3.3. Effect of extraction method and organ on the anti-radical activity against synthetic radical DPPH

DPPH radical scavenging capacity of our plant extract samples was investigated at a concentration of 30 mg/ml. As shown in Figure 1, all samples exhibited high antioxidant activity against DPPH as the scavenging activities of the different samples were ranging from 32.06% (for fresh bulbs extracted by decoction) to 66.61% (for fresh bulbs extracted by infusion). These results are important since half maximal inhibitory concentration (IC<sub>50</sub>) values are low. It was recalled that the lower IC<sub>50</sub> indicates the higher free radical-scavenging ability. For example, for fresh bulbs extracted by infusion, IC<sub>50</sub> was lower than 30 mg/ml.

Besides, our data regarding the antioxidant activity of infusion and decoction extracts from fresh bulbs and leaves highlighted the variability of antioxidant potential depending on extraction methods and organ (Fig. 1).

Synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), t-butylhydroquinone (TBHQ) and propyl gallate, have been widely used in food products to delay the

deterioration caused by lipid oxidation. However, these antioxidants create potential health hazards, and their use has been restricted in many countries. Thus, it is essential to develop safe and natural antioxidants as alternatives to synthetic ones. For that purpose, antioxidant properties of fresh bulbs and leaves of *A. ursinum* extracted using the two methods (infusion and decoction) were assessed using the DPPH radical-scavenging assay.



**Figure 1.** Variability of inhibition percentage of DPPH with extraction method and fresh organ of *A. ursinum*.

Free radical-scavenging is a primary mechanism by which antioxidants inhibit oxidative processes. The DPPH radical scavenging assay is a widely used method for evaluating the ability to scavenge free radicals generated from DPPH reagent. DPPH is a stable free radical, which can be reduced by a proton-donating substrate such as an antioxidant, causing the decolorization of DPPH and the reduction of the absorbance at 517 nm. The decrease in absorbance is taken as a measure for radical scavenging activity. Our results are in agreement with those of Sapunjieva et al. (2012) who proved that the extraction method affects significantly the ability to neutralize reactive oxygen species (ROS). However, there is no direct correlation with phenolic composition. In fact, the extract from leaves obtained by decoction, which corresponds to the high value of polyphenols (5.68 mg GAE/g FP) did not represent the most active extract against DPPH (33.95 %). Moreover, study of Mivaylova et al. (2014) confirmed that polyphenolic content and antioxidant activity have been successfully correlated. This variability could be attributed to the presence of particular compounds extracted by specific method which are responsible for the scavenging activity. This can explain the presence, in our study, of two sulfur compounds (Disulfide, methyl propyl and Disulfide, dipropyl) in the most active extract against DPPH (extract obtained by infusion from bulbs).

Regarding the change in the anti-radical activity according to the organ of plant, our results are similar to those of Sapunjieva et al. (2012) and those of Maisuthisakul et al. (2007) and confirm that the distribution of antioxidant metabolites differ from one organ to another in the same plant, due to their specificity (Ksouri et al, 2009; Falleh et al, 2009).

#### 4. Conclusion

*A. ursinum*, a wild species of *Allium* genus, has since ancient times a great popularity. It is widely used in alternative medicine and credited with antioxidant properties. In this study, biochemical composition and antioxidant activity were investigated on fresh bulbs and leaves of Tunisian *A. ursinum* extracted using two different methods, namely infusion and decoction. Our findings showed that both organ and extraction method significantly affected biochemical composition and antioxidant activity. These variabilities were mainly due to their chemical composition which qualitatively and quantitatively dependent on the organ and the extraction method. However, the significance of this effect must be investigated further. Nevertheless, the obtained results suggested that *A. ursinum* bulbs and leaves extracts could be applied as proton donors and could react with free radicals to convert them to more stable products and terminate the radical chain reaction.

## 5. References

- Aaby, K., Hvattum, E., Skrede, G (2004).** Analysis of flavonoids and other phenolic compounds using high-performance liquid chromatography with coulometric array detection: Relationship to antioxidant activity. *J Agric Food Chem* 52: 4595–4603.
- Agarwal, KC (1996).** Therapeutic action of garlic constituents. *Med Res Review* 16: 111-124.
- Augusti, KT (1977).** Hypocholesterolemic effect of garlic, *Allium sativum* Linn. *J Exp Biol* 15: 489-490.
- Bettaieb Rebey, I., Bourgou, S., Ben Slimen Debez, I., Jabri Karoui, I., Hamrouni Sellami, I., Msaada, K., Limam, F., Marzouk, B (2012).** Effects of extraction solvents and provenances on phenolic contents and antioxidant activities of Cumin (*Cuminum cyminum* L.) seeds. *Food Bioprocess Technol* 5: 2827–2836.
- Błażewicz-Woźniak, M., Michowska, A (2011).** The growth, flowering and chemical composition of leaves of three ecotypes of *Allium ursinum* L. *Acta Agro* 64: 171-180.
- Bersuder, P., Hole, M., Smith, G (1998).** Antioxidants from a heated histidine-glucose model system. I: Investigation of the antioxidant role of histidine and isolation of antioxidants by high-performance liquid chromatography. *J Amer Oil Chem Soc* 75: 181-187.
- Djurdjevic, L., Dinic, A., Pavlovic, P., Mitrovic, M., Karadzic, B., Tesevic, V (2003).** Allelopathic potential of *Allium ursinum* L. *Biochem Syst Ecol* 32: 533-544.
- Dewanto, V., Wu, X., Liu, RH (2002).** Processed sweet corn has higher antioxidant activity. *J Agric Food Chem* 50: 49, 59-64.
- Do, QD., Angkawijaya, AE., Tran-Nguyen, PL., Huynh, LH., Soetaredjo, FE., Ismadji, S., Ju, YH (2013).** Effect of extraction solvent on total phenol content, total flavonoids content, and antioxidant activity of *Linnophila aromatica*. *Journal of food and drug analysis* xxx: 1-7.
- Falleh, H., Ksouri, R., Oueslati, S., Guyot, S., Magné, C., Abdelly, C (2009).** Interspecific variability of antioxidant activities and phenolic composition in *Mesembryanthemum* genus. *Food Chem Toxicol* 47: 2308-2313.
- Gîtin, L., Dinica, R., Parnavel, R (2012).** The influence of extraction method on the apparent content of bioactive compounds in romanian *Allium spp.* Leaves. *Not Bot Horti Agrobo Cluj-Napoca* 40: 93-97.
- Godevac, D., Vujisic, L., Mojovic, M., Ignjatovic, A., Spasojevic, I., Vajs V (2008).** Evaluation of antioxidant capacity of *Allium ursinum* L. volatile oil and its effect on membrane fluidity. *Food Chem* 107: 1692-1700.
- Hayouni, EA., Abedrabba, M., Bouix, M., Hamdi, M (2007).** The effects of solvents and extraction method on the phenolics contents and biological activities in vitro of Tunisian *Quercus coccifera* L. and *Juniperus phoenicea* L. fruit extracts. *Food Chem* 105: 1126–1134.
- Jalleli, I., Megdiche, W., Hamdi, M., Oueslati, S., Smaoui, A., Abdelly, C., Ksouri, R (2012).** Changes in phenolic composition and antioxidant activities of the edible halophyte *Crithmum maritimum* L. with physiological stage and extraction method. *Acta Physiol Plant* 34: 1451–1459.
- Ksouri, R., Falleh, H., Megdiche, W., Trabelsi, N., Mhamdi, B., Chaieb, K., Bakrouf, A., Magné, C., Abdelly, C (2009).** Antioxidant and antimicrobial activities of the edible medicinal halophyte *Tamarix gallica* L. and related polyphenolic constituents. *Food Chem Toxicol* 47: 2083-2091.
- Mathew B (1996).** A Review of *Allium* section *Allium*. *Royal Botanic Gardens, Kew*. Richmond, Surrey, UK.
- Milane H (2004).** La quercétine et ses dérivés: molécules à caractère pro-oxydant ou capteurs de radicaux libres; études et applications thérapeutiques. Louis Pasteur, Strasbourg, France, 13-36.
- Maisuthisakul P., Pongsawatmanit R., Gordon M H (2007).** Assessment of phenolic content and free radical scavenging capacity of some Thai indigenous plants. *Food Chem* 100: 1409-1418.
- Mivaylova, DS., Lante, A., Trinello, F., Krastanov, AI (2014).** Study on the antioxidant and antimicrobial activities of *Allium ursinum* L. pressurized-lipoid extract. *Nat Prod Res* 28: 2000-20005.
- Rangsrivong, P., Rangkadilok, N., Satayavivad, J., Goto, M., Shotipruk, A (2009).** Subcritical water extraction of phenolic compounds from *Terminalia chebula* retz fruits. Separation and purification technology 66: 51-56.

- Ouerdraogo RA, Koala M, Dabire C, Hema A, Bazie V, Ouattara LP, Gnoula C, Pale E, Nebie R (2015).** Teneur en polyphénols totaux et activité antioxydante des extraits des trois principales variétés d'oignons (*Allium Cepa L.*) cultivées dans la région du Centre-Nord du Burkina Faso. Inter J Biotech ChemSci 9: 281-291.
- Oszmiański, J., Kolniak-Ostek, J., Wojdyło, A (2013).** Characterization and content of flavonol derivatives of *Allium ursinum L.* Plant. J Agric Food Chem 61: 176-184.
- Phillips R, Rix M (1998).** Conservatory and indoor plants. Vol. 1, Macmillan. (Ed). London UK.
- Preuss, H., Clouatre, D., Mouhamadi, A., Taylor-Jarrel, S (2001).** Wild garlic has a greater effect than regular garlic on blood pressure and blood chemistries of rats. Inter Urol Nephrol 32: 525-530.
- Qidwai W, Ashfaq T (2013).** Role of garlic usage in cardiovascular disease prevention: An evidence-based approach. Evid Based Complem Alter Med. 1-9.
- Quispe Candori, S., Foglio, MA, Rosa PTV, Meireles, MAA 2008.** Obtaining b-caryophyllene from *Cordia verbenacea* de Candolle by super critical fluid extraction. J. Supercrit. Fluids 46: 27–32.
- Stearse, SE., Yellon, DM (1995).** The potential for endogenous myocardial antioxidant to protect the myocardium against ischemic-reperfusion injury: refreshing the parts exogenous antioxidants cannot reach. J Mol Cell Cardiol 27: 65-74.
- Stalikas, CD (2007).** Extraction, separation, and detection methods for phenolic acids and flavonoids. J Sep Sci 30: 3268-95.
- Sapunjeva, T., Alexieva, I., Mihaylova, D., Popova, A (2012).** Antimicrobial and antioxidant activity of extracts of *Allium ursinum L.* J Bio Sci Biotech 143-145.
- Sobolewska, D., Podolak, I., Makowska Was, J (2015).** *Allium ursinum*: botanical, phytochemical and pharmacological overview. Phytochem Rev 14: 81-97.
- Sun, B., Wang, W., Salvaterra, P (1998).** Functional analysis and tissue-specific expression of Drosophila Na<sup>+</sup>,K<sup>+</sup>-ATPase subunits. J Neuro Chem 71: 142-151.
- Turkmen, N., Sari, F., Sedat, Velioglu, Y (2006).** Effects of extraction solvents on concentration and antioxidant activity of black mate tea polyphenols determined by ferrous tartrate and Follin-ciocalteu methods. Food chem 99: 835-841.
- Vasile Bagiu, R., Vlaicu, B., Butnariu, M (2012).** Chemical composition and in vitro antifungal activity screening of the *Allium ursinum L. (Liliaceae)*. Inter J Mol Sci 13: 1426-1436.
- Zhishen, J., Mengcheng, T., Jianming, W (1999).** The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem 64: 555-559