A comparative study of the phytochemical, antioxidant and anti-inflammatory properties of ethyl acetate extract of *Asphodelus tenuifolius* Cav recovered by different extraction techniques

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ABSTRACT

In this study, phytochemical, in vitro antioxidant activities and anti-inflammatory of bioactive components of *Asphodelus tenuifolius* Cav extracts by classical extraction method (CE), Soxhlet extraction (SE), ultrasonic extraction (UE) and microwave extraction (ME) were investigated. The total phenolic, flavanoids, condensed tannins and anthocyanin content were determined by a spectrophotometric method. β-Carotene, ABTS, O_2^- and FRAP radical-scavenging were applied to test the antioxidant activities. The results indicated that the classical method extraction exhibited the strongest antioxidant activities. The contents of polyphenols in the CE were significantly higher than those by other extraction methods, which was possibly responsible for the higher antioxidant activities of the *Asphodelus tenuifolius* Cav by CE. From the results we can draw the conclusion that not only the more bioactive components are obtained but also the extract has better free radical and reactive oxygen species scavenging activities through classical extraction method. These findings further illustrate that classical extraction has a bright prospect for extracting active ingredients from plant materials. The study concludes that *Asphodelus tenuifolius* Cav possess diverse therapeutic potentials which might be useful in development of drugs or their precursors.

KEYWORDS: *Asphodelus tenuifolius* Cav**,** extraction techniques, Phytochemical, Antioxidant activity, anti-inflammatory.

1. Introduction

Natural antioxidants, which are ubiquitous in medicinal plants, have received great attention and have been studied extensively, since they are effective free radical scavengers and are assumed to be less toxic than synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxyltoluene (BHT), which are suspected of being carcinogenic and causing liver damage [1, 2]. Natural products have long been used to prevent and treat cancer and thus they are good candidates for the development of anti-cancer drugs [3, 4]. Recent epidemiological studies have indicated that diets rich in fruits, vegetables and those of selected plant compounds are correlated with reduced incidence of cardiovascular and chronic diseases like cancer [5]. The phenolic compounds or polyphenols, secondary vegetal metabolites, constitute a wide and complex array of phytochemicals that exhibit antioxidant action and consequently a beneficial physiological effect [6, 7]. Their ability to delay lipid oxidation in foodstuffs and biological membranes, in addition to their propensity to act as a prophylactic agent has motivated research into food science and biomedicine [8]. Considering their bioactivity and their presence in a wide range of vegetables, these substances are considered natural antioxidants and the vegetable source that it contains as functional food.

These compounds appear to a play a significant role as antioxidants in the protective effect of plant derived foods [9]. Phenolics have become the focus of current nutritional and therapeutic interest. The antioxidant activity of the dietary phenolics is considered to be superior to that of the essential vitamins and is ascribed to its high redox potential which allows them to interrupt free radical mediated reactions by donating hydrogen from the phenolic hydroxyl groups [10]. Furthermore, some plant constituents such as anthocyanin, and condensed tannins have also been documented to exhibit various biological activities including anti-inflammatory, antiatherosclerotic, antitumor, antimutagenic, anticarcinogenic, antibacterial and antiviral activities [11, 12]. However, majority of these plants have not been investigated for their antioxidant potency.

The genus *Asphodelus tenuifolius* Cav belongs to the Liliaceae family, which comprises 187 genera and 2500 species. This species distributed in North African, Southern Europe, India, and Pakistan. Various species of this genus are used as anti-ulcer and anti-inflammatory agents, and also as a diuretic and for the prevention of atherosclerosis [13]. The ethnopharmacological and chemotaxonomic importance of the *Asphodelus tenuifolius* Cav prompted us to carry out phytochemical studies on one of its species. It is a small erect annual herb, which commonly grows in different areas of Southeast of Algeria [9]. Literature survey revealed that no phytochemical or pharmacological studies have so far been carried out on this species. The objectives of this work were to investigate the phytochemical composition, antioxidant and anti-inflammatory activity of natural extracts of *Asphodelus tenuifolius* Cav recovered by different extraction techniques and as consequence to exploit its potential as a natural preservative.

2. Materials and methods

2.1. Collection and processing of plant sample

The areal part *Asphodelus tenuifolius* Cav were collected from the El Oued regions of the North-Est Algeria (33° 07" North and 7° 11" Est) in the month of Mai 2013. The aerial part was dried to a constant weight in the oven at 45 ◦C for 24–48 h, grated and reduced to powder using a laboratory miland and stored in amber-coloured bottles at ambient conditions until use [14, 15]. For this work, ethyl acetate extracts of aerial part of *Asphodelus tenuifolius* Cav were used for the determination of antioxidant activity, phytochemical investigation, anti-inflammatory property and antifungal capacity.

2.1.1. Classical extraction (CE)

Fifteen grams of powdered leaves (15 g) and ethyl acetate (150 mL) were put in a series of the Erlenmeyer flasks (250 mL), and the ratio of plant material mass (g) to solvent volume (mL) was 1:10. No additional stirring was applied. The extraction was performed at 30 °C for 60 min. At the end of the extraction cycle the liquid extract was separated from the solid residue by vacuum filtration. The solid residue was washed twice with fresh solvent (20 mL). The filtrates were collected and the solvent was evaporated in a rotary vacuum evaporator at 45 °C. Extracts were stored at +4 °C in dark until use

2.1.2. Ultrasonic-assisted extraction (UAE)

Ultrasonic apparatus from Branson (40 kHz, 1500W, dimension: 49 cm×14 cm×15 cm) was used for accelerated extraction. A beaker was partially submerged in an isothermal water bath to maintain the extraction temperature at 30 °C. Fifteen grams (15 g) were then extracted with ethyl acetate (100 mL) for 60 min.. The extract was filtered through Whatman No. 4 paper on a Büchner funnel by vacuum; the solids were washed with an additional 50 mL of ethyl acetate. The filtrate was rotary-evaporated under vacuum at 40 ◦C to dryness [16]. The crude extracts were dried in a vacuum oven at 45 ◦C. Extracts were stored at +4 °C in dark until use.

2.1.3. Soxhlet extraction (SE)

Fifteen grams of powdered were mixed with 150 mL ethyl acetate and extracted in a Soxhlet apparatus for 6 h. The extracts concentrated under vacuum at 40 °C by using a rotary evaporator. To obtain ethyl acetate extracts, air dried powdered plants were boiled with 250 mL of ethyl acetate for 30 min [17]. The ethyl acetate extracts were filtered and concentration using rotary-evaporated under vacuum at 45 ◦C to dryness. Extracts were stored at +4 °C in dark until use.

2.1.4. Microwave assisted extraction (MAE)

Fifteen grams of powders were mixed with ethyl acetate of 300 mL in microwave extraction vessel (Xt-9912a, Xi'an Yima Opto-Electrical Technology Co., Ltd. Beijing, China). All the microwave extractions were performed under a set microwave irradiation power (1600 W) for a certain period of time (10 min). During the process of extraction, the powder-solvent mixture was stirred at

regular intervals to ensure homogenous exposure of the mixture to microwave irradiation [18]. The filtrates were collected as phenolic extracts. The extract was filtered through Whatman No. 4 paper on a Büchner funnel by vacuum and were reduced to a small volume by rotary evaporation under vacuum at 45 °C and stored at +4 °C in dark until use.

2.2. Phytochemical screening

2.2.1. Total phenolics

Total phenolic content of each extract method was determined with the Folin–Ciocalteu's reagent (FCR) [19, 20]. Briefly, a dilute solution of each extract in ethyl acetate (1 mL) was mixed with 1 mL of folin-ciocalteu reagent, followed by 1 mL of a $CaCO_3$ (10 % w/v) after 4 min. The reaction mixture was incubated for 30 min at room temperature. The absorbance of reaction mixture at 700 nm was calculated, the blank's prepared with the same procedure described above except that the samples solution was substituted by 1 mL of ethyl acetate. The concentration of total phenolic in the extracts was expressed as mg gallic acid equivalent (GAE) per g of dry weight. All results presented are means (±SEM) and were analyzed in three replications.

2.2.2. Total flavanoids

Flavonoid content was determined by using a method described by Ardestani et al. [21]. Each sample (0.5 mL) was mixed with 2 mL of distilled water and subsequently with 0.15 mL of a $NaNO_2$ solution (15%). After 6 min, 0.15 mL of aluminum chloride ($AlCl_3$) solution (10%) was added and allowed to stand for 6 min, then 2 mL of NaOH solution (4%) was added to the mixture. Immediately, water was added to bring the final volume to 5 ml and the mixture was thoroughly mixed and allowed to stand for another 15 min. Absorbance of the mixture was then determined at 510 nm versus prepared water blank. Results were expressed as catechin equivalent (mg CE/g dried extract). All measurements were made in triplicate and averaged.

2.2.3. Total condensed tannins (proanthocyanidins)

The proanthocyanidins content was determined using a spectrophotometric method [22]. A volume of 0.5 mL of ethyl acetate extract or standard (catechin) was added to the mixture of 3 ml of 4% vanillin- methanol (4%, v/v), 1.5 mL of hydrochloric acid and then vortexed. The resulting mixture was allowed to stand for 15 min at room temperature, the absorbance of each was measured at 500 nm using spectrophotometer (Shimadzu UV-1800, Japan). Total proanthocyanidin content was calculated as mg catechin equivalent (mg CTE/g). All measurements were made in triplicate and averaged.

2.2.4. Total anthocyanin

The total anthocyanin content of the ethyl acetate extract of each method was evaluated using spectrophotometer (Shimadzu UV-1800, Japan) by the pH-differential method [23, 24]. Two buffer solution: KCl buffer, pH 1.0 (0.025 M) and sodium acetate buffer, pH 4.5 (0.4 M). A volume of 3.6 ml for corresponding buffers added to 0.4 ml of ethanolic extract or standard (cyanidin-3-glucoside) and read against a blank at 510 and 700 nm. Absorbance (A) was calculated using the following equation:

$\Delta A = (A510 - A700)_{pH\ 1.0} - (A510 - A700)_{pH\ 4}$. Monomeric anthocyanin pigment concentration in the extract was estimated as cyanidin-3-glucoside (mg/l) = $A \cdot MW \cdot DF \cdot 1000 / (MA \cdot 1)$.

Where A: absorbance; MW: molecular weight (449.2); DF: dilution factor; MA: molar absorptivity (26,900). The total anthocyanin was expressed as milligrams of cyanidin-3-glucoside per g of dry Weight (mg/g).

2.3. Antioxidant activity

2.3.1. β-Carotene linoleic acid bleaching assay

The antioxidant capacity is determined by measuring the inhibition of the production of volatile organic compounds and the formation of conjugated diene hydroperoxides arising from linoleic acid

oxidation [25]. Firstly the β-Carotene was prepared by dissolving 2 mg of this reagent in 10 ml of chloroform. In the round-bottom flask are added 2 mL of β-Carotene solution, 40 mg of linoleic acid and 400 mg of Tween 80, after this preparation, the chloroform is removed at 40 °C using the rotary evaporator). The resulted mixture was added to 100 mL of distilled water (aerated) under vigorous shaking and protection from the light. A 4.8 mL of the last solution was transferred into different tube containing 0.2 mL of each extracts method in ethyl acetate with different concentrations. A control sample was prepared of 0.2 mL ethyl acetate and 4.8 mL of β-Carotene reagent. The tubes were incubated at 50 °C for 2 h. the absorbance at 470 was measured, using UV-Visible spectrophotometer [26, 27]. The essay was carried out in triplicate and the results were provided as 50 % inhibition (EC_{50} µg/ml). The antioxidant activity was calculated using the following equation:

$$\% \text{ antioxidant activity} = ((A_o - A_t)/ (A_o° - A_t°)) \times 100 \quad (1)$$

Where, A_o and A_t are respectively the absorbance calculated at zero time of incubation for simple extracts and control. $A_o°$ and $A_t°$ are the absorbance measured after 2 h respectively for simple extract and control. All measurements were made in triplicate and averaged.

2.3.2. Scavenging activity of superoxide radicals

The superoxide anion scavenging of each extracts method was estimated using the inhibition of NBT reduction by photochemical generated O_2^-. To the assay mixture contained 2 µM of riboflavin, we added 6 µM EDTA, 50 µM NBT and 3 µg of sodium cyanide in 67 mM phosphate buffer (pH= 7.8) in a final volume of 3 mL. Initial absorbance was measured at 530 nm, the tubes were illuminated uniformly with incandescent lamp at 530 nm. The sample extract was added to the reaction mixture, in which O_2^- radicals are scavenged, thereby inhibition the NBT reduction [28]. Quercetin used as a positive control and the percentage of scavenging inhibition was calculated as:

$$\% \text{ inhibition} = [(A_{control} - A_{sample})/A_{control}] \times 100 \quad (2)$$

All measurements were made in triplicate and averaged.

2.3.3. Ferric reducing antioxidant power (FRAP assay)

The reducing power was determined by using FRAP assay [29]. Briefly, the FRAP reagent contained 2.5 mL of 10 mM tripyridyltriazine (TPTZ) in 40 mM HCl, 2.5 mL of 20 mM $FeCl_3$ and 25 mL of 0.3M acetate buffer (pH 3.6), was freshly prepared. A volume 0.2 of ethanolic extract (various concentrations) or standard was mixed with 1.8 mL of freshly prepared FRAP reagent . The absorbance of each sample solution was subsequently measured at 595 nm. For the calibration curve, $FeSO_4$ was prepared in ethyl acetate in the range of 100–700 µM and Quercetin was used as positive controls. The results were expressed as mg/ml of Fe(II), using the equation obtained from the calibration curve of $FeSO_4$: $Y = 6.908x + 0,028$, $R^2 = 0.998$. Each sample was performed in triplicate.

2.3.5. Metal chelating activity

The chelating of ferrous ions by the each extracts methods and standards was evaluated by the method reported by Wen et al. [30]. 2.5 mL of each extract method (different concentration) were respectively mixed with 0.05 mL of 2 mmol/L $FeCl_2$ solution. The reaction was initiated by the addition of 0.2 mL of 5 mmol/L ferrozine and the mixture was shaken vigorously and left standing at room temperature for 10 min. After the mixture had reached equilibrium, the absorbance of the solution was then measured at 562 nm in a spectrophotometer. All measurements were made in triplicate and averaged. The cheating activity on Fe^{2+} was calculated using the following equation: Cheating activity on Fe^{2+} (%) = [(1 – Absorbance of sample at 562 nm)/Absorbance of control at 562 nm] × 100. EC_{50} (mg/mL) is the concentration at which the cheating activity on Fe^{2+} was 50%. Each sample was performed in triplicate.

2.4. Nitric oxide generation and determination by Griess reagent

Nitric oxide was produced from sodium nitroprusside. It interacts with oxygen to produce nitrite ion and determined by the use of Griess reagent [31]. A volume of 2 mL of sodium nitroprusside prepared in saline phosphate buffer (pH= 7.4) was added to 0.5 mL of different concentrations of plant extracts, BHT and querecetin. The mixture was set at 25 °C for 150 min. 0.5 mL of each sample from above solutions were added to 0.5 mL of Griess reagent (1% sulphanilamide, 2% H_3PO_4 and 0.1% ACS reagent) and allowed to stand for 30 min. The absorbance of the chromophore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with naphtylethylene diamine was measured at 546 nm. The amount of nitric oxide radicals was calculated using the equation 2.

2.5. Statistical analysis

All analyses were carried out in triplicates. Data were presented as mean ± standard deviation (SD). Statistical analyses were performed by one-way ANOVA. Significant differences between groups were determined at $P < 0.05$. To evaluate relationships between experimental parameters, results were analyzed for correlation and regression and tested for significance ($P < 0.05$).

3. Results and discussion

3.1. Total phenolic, flavanoids, condensed tannins and anthocyanin

The extracts technique methods of aerial parts of *Asphodelus tenuifolius* Cav differ to each other considerably with respect to the total phenolic, flavonoid, condensed tannins and anthocyanin as it can be seen in Table 1. The total phenolic content of different extraction techniques ranges from (124.27±1.9) mg GAE /g to (151.7±3.5) mg GAE /g, the content of flavanoids in catechin equivalent, varies from (92.8±1.4) mg CE /g to (116.5±2.5) mg CE /g, condensed tannins expressed in catechin equivalent varies from (62.8±0.4) mg CE /g to (86.5±1.2) mg CE /g, while content anthocyanin expressed in cyanidin-3-glucoside per g of dry weight varies from (88.7±1.4) mg cyanidin-3-glucoside E/g to (115.8±2.1) mg cyanidin-3-glucoside E/g. The highest amounts of total phenolic compound, flavanoids, condensed tannins and anthocyanin are found in the extracts obtained by classical and ultrasonic extraction and the lowest amounts is obtained by the SE and ME method. Ultrasonic is governed by mechanical and chemical effects of acoustic cavitation collapse, resulting in the disruption of cell walls, which facilitates the release of their content into the system, and producing high local pressures and temperatures [32]. This may explain the phenolic content increase of twig extract by the UE method. However this phenomenon (local high temperature and high pressure produced by cavitation collapse) could induce the degradation of unstable compounds. This may be explained by oxidation and degradation of these bioactive compounds under the higher extraction temperature and the much longer extraction time of the Soxhlet extraction [33, 34]. The extraction method has a statistically significant influence on the total phenolic, flavonoid, condensed tannins and anthocyanin content in the extracts.

Table 1: Total phenolic, flavonoid, condensed tannins and anthocyanin content

Extraction technique	Phenolic content mg GAE /g	Flavanoid content mg CE/g	Condensed tannins mg CE/g	Anthocyanin mg cyanidin-3-glucoside E /g
CE	151.7±3.5	116.5±2.5	86.5±1.2	115.8±2.1
UE	140.27±3.1	104.2±2.1	79.13±1.1	103.2±1.7
SE	126.46±2.5	99.5±1.2	70.46±0.8	96.5±1.2
ME	124.27±1.9	92.8±1.4	62.8±0.4	88.7±1.4

Note: CE- Classical extraction, UE- Ultrasonic extraction, SE- Soxhlet extraction, ME- Microwave extraction.

3.2. Antioxidant capacities

3.2.1. β-Carotene

In β-Carotene linoleate model system free radical arises from oxidation of linoleic acid, attacked by the highly unsaturated β-Carotene molecules and causing decrease in absorbance at 470 nm. The presence of different antioxidants can hinder the extent of β-Carotene blanching by neutralization of the linoleate-free and other free radicals formed in the system [35]. The results of inhibition activity (50 %) of extracts and the positive control (BHT) were showed in Table 2. The interaction of a potential antioxidant with β-Carotene depends on the extraction technique. The following order of antioxidant activity is established: extracts obtained by classical extraction>extracts obtained by ultrasound extraction>extracts obtained by Soxhlet extraction> extracts obtained by microwave extraction.

The results indicated and supported that the presence of phenolic content with high concentration in the extracts of can moderately prevent the degradation of β-Carotene caused by radical reactions. Thus, consumption of such underutilization of the antioxidant can protect the oxidation and degradation of cellular macromolecules due to free-radical attacks. The amount of phenolic compounds in the extracts correlates with their antioxidant activity phenolic compound confirming that these compounds are likely to contribute to the radical scavenging activity of these plant extracts.

3.2.2. Superoxide radicals

The assay was based on the capacity of different extracts techniques methods of *Asphodelus tenuifolius* Cav. to enhance the formation of formazane in comparison to the NBT/riboflavin reference signal. The results of extracts scavenger represented in Figure 1. The following order of antioxidant activity is established: extracts obtained by classical extraction>extracts obtained by ultrasound extraction>extracts obtained by Soxhlet extraction> extracts obtained by microwave extraction. The higher inhibition are found in the extract by CE (IC_{50}= 0.346 ± 0.02 mg/mL), UE (IC_{50}=0.429 ± 0.07 mg/mL), SE (IC_{50}=0.481 ± 0.09 mg/mL), while ME displays the lowest response in this assay (IC_{50}= 0.648 ± 0.09 µg/mL).

Table 2: β-Carotene bleaching activities of methanol extracts of *Asphodelus tenuifolius* Cav and of authentic standards (BHA and BHT). Antioxidant activity was expressed as % inhibition IC_{50}

Extraction technique	Samples concentrations (mg/ml)	Reaction time (min)		Antioxidant activity IC_{50}
		30	120	
CE	0.1	09.56 ± 0.08	4.32 ± 0.11	0.449±0.003 (mg/ml)
	0.3	78.45 ± 2.67	31.35 ± 1.82	
	0.5	85.32 ± 3.68	56.97 ± 2.43	
UE	0.1	11.32 ± 0.04	02.40 ± 0.29	0.502± 0.002 (mg/ml)
	0.3	53.55 ± 2.01	23.70 ± 1.02	
	0.5	85.68 ± 3.18	50.31 ± 1.85	
SE	0.1	04.21 ± 0.02	00.92 ± 0.06	0.583 ± 0.005 (mg/ml)
	0.3	56.61 ± 2.02	17.31 ± 1.03	
	0.6	86.23 ± 3.45	52.09 ± 1.05	
ME	0.1	4.45 ± 0.030	00.34 ± 0.05	0.601 ± 0.007 (mg/ml)
	0.3	28.65 ± 1.06	13.86 ± 1.04	
	0.6	82.12 ± 2.45	50.34 ± 2.21	
BHT	0.01	0 ± 0	0 ± 0	91.34 ± 3.76 (µg/ml)
	0.03	03.78 ± 0.02	01.23 ± 0.04	
	0.05	80.21 ± 3.14	55.35 ± 2.37	

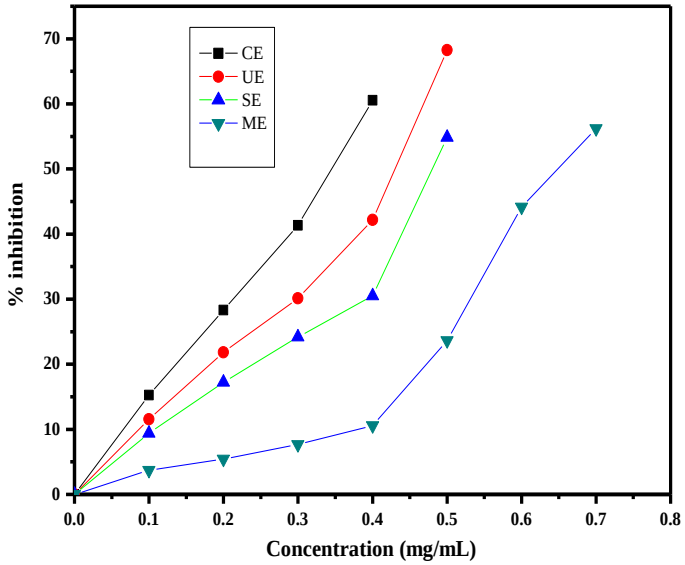

Figure 1: Superoxide scavenging potential of different extracted technique of *Asphodelus tenuifolius* Cav in photoreduction assay measured by NBT method..

3.2.3. Metal chelating activity

CE, UE, SE and ME were assessed for their ability to compete with ferrozine for Fe^{2+} in free solution. All the extracts demonstrated an ability to chelate Fe^{2+} in a dose-dependent manner (Figure 2). CE showed a much higher chelating ability (IC_{50}=0.353 ± 0. 05 mg/mL). The chelating effect of UE, SE and ME respectively (IC_{50}=0.434 ± 0.02 mg/mL), (IC_{50}=0.473 ± 0.08 mg/mL) and (IC_{50}=0.501 ± 0.0 8mg/mL). The superoxide radical-scavenging assay and was performed to determine the abilities of the extracts to inhibit oxidation. The CE and UE methods exhibited the strongest antioxidant activities. The antioxidant activities of extracts by CE and UE methods are in accordance with the amount of phenolic compounds.

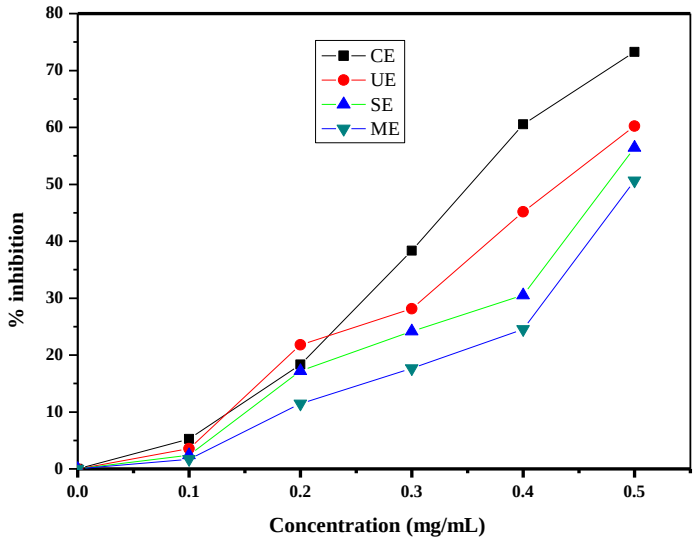

Figure 2: Chelating activity of the extracts from *Asphodelus tenuifolius* Cav recovered by different extraction methods.

3.2.4. Nitric oxide generation

The scavenging activity of the extracts by different methods against nitric oxide were calculated. All extracts down-regulated NO production with IC_{50} < 0.5 mg/mL. The strongest effect was observed for the CE with an IC_{50}= 240.28 ± 8.42 mg/mL. Regarding the other extracts methods UE IC_{50}= 307.89 ± 11.25 mg/mL, SE IC_{50}= 390.72 ± 13.15 mg/mL and ME, The results were shown in figure 3.

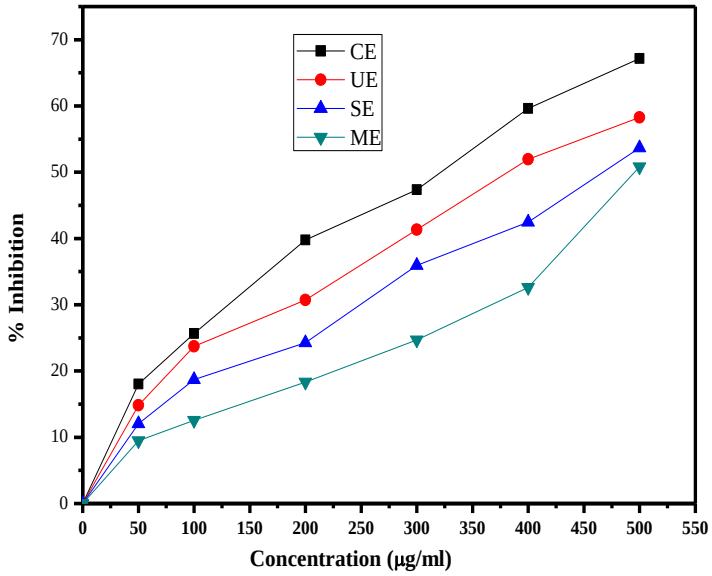

Figure 3: Nitric oxide scavenging activities of the extracts from *Asphodelus tenuifolius* Cav by different CE, UE, SE and ME methods

Numerous plants rich on phenolic compounds have been investigated as potential inhibitors of NO production in inflammatory reaction [36]. These compounds used in the treatment of chronic inflammatory diseases associated with overproduction of nitric oxide [37]. The toxicity of nitric oxide increases greatly when it reacts with superoxide radicals forming the highly reactive peroxynitrite anion ($ONOO^-$) [38]. The nitric oxide generated from sodium nitroprusside reacts with oxygen to form nitrite. The leaves ethanolic extract of the three varieties inhibits nitrite formation by directly competing with oxygen in the reaction with nitric oxide. The present study proved that the leaves extract have good nitric oxide scavenging activity.

4. Conclusion

The results of the present study demonstrate that different methods of extraction patterns of active components in the extracts. In vitro antioxidant activities and bioactive components of *Asphodelus tenuifolius* Cav by different extraction methods CE, UE, SE and ME have been evaluated. The results indicated that *Asphodelus tenuifolius* Cav extract by classical method exhibited strongest antioxidant activities after respectively ultrasonic, Soxhlet and microwave methods. The contents of phenolic, flavanoids, condensed tannins and anthocyanin in the classical extract were significantly higher than those by other extraction methods, which were possibly responsible for higher antioxidant and anti-inflammatory activities CE. From the results we can draw the conclusion that not only the more bioactive components are obtained but also the extract has better free radical, reactive oxygen species and nitric oxide scavenging activities through classical method. These findings further illustrate that CE has a bright prospect for extracting active ingredients from plant materials.

References

[1] Ratnam D. V., Ankola D. D., Bhardwaj V., Sahana D. K. and Kumar R. M. N. V. ; *J. Controlled Release* **113**, 189–207(2006).
[2] Ke-XueZ., Cai-Xia L., Xiao-Na G., Wei P. and Hui-Ming Z.; *Food Chemistry* **126**, 1122–1126 (2011).
[3] Smith-Warner S. A., Elmer P. J., Tharp T. M., Fosdick L., Randall B., Gross M., Wood J. and Potter J. D.; *Cancer Epidemiol. Biomar. Prev* **9** (3), 307–317 (2000).
[4] Rajkumar V., Gunjan Guha R. and Ashok K.; *Food Chem. Toxicol.* **49**, 363–369 (2011).
[5] Perez-Jimenez J. and Saura-Calixto F.; *J. Agric. Food Chem.* **53**, 5036–5040 (2005).
[6] Enayde de Almeida M., Jorge Mancini F. and Nonete Barbosa G.; *Lebensm.-Wiss. u.-Technol*. **38**, 15–19 (2005).
[7] Martinez-Valverde I., Periago M J. and Ros G.; *Arch Latinoam Nutr* **50**, 5–18 (2000).
[8] Farombi E. O., Britton G. and Emerole G. O.; *Food Res. Int.* **33**, 493–499 (2000).
[9] Muhammad S., Muhammad I., Rashad M., Abdul M., Nighat A., Lubna I. and Mehreen L.; J. Asian Nat. Prod. Res. **11**, 945–950 (2009).
[10] Chandha S.Y.R.; The Wealth of India, A dictionary of Indian raw materials and industrial products (CSIR, Niscair Press, New Delhi, 2003), Vol. I, p. 472.
[11] Papi A., Orlandi M., Bartolini G., Barillari J., Iori R. and Paolini M.; *J. Agric Food Chem* **56**,8750–8883 (2008).
[12] Beevi S.S., Mangamoori L.N., Dhand V. and Siva Ramakrishna D.; *Foodborne Pathog. Dis.* **6**, 129–136 (2009).
[13] Baquar S. R.; Medicinal and Poisonous Plants of Pakistan, 1st ed. (Karachi Printing Press, Karachi), p 45 (1989).
[14] Doughari J. H., Ndakidemi P. A., Human I. S. and Benade S.; *J. Ethnopharmacol* **141** (3), 1041–1050 (2012).
[15] Doughari J. H., El-mahmood A. M. and Tyoyina I.; *Afr J. Pharmacy Pharmacol* **2**, 07–013 (2008).
[16] Diouf P. N., Stevanovic T. and Boutin Y.; *Ind. Crop. Prod.* **30**, 297–303 (2009).
[17] Aktumsek A., Zengin G., Guler G. O., Cakmak Y. S. and Duran A.; *Food Chem. Toxicol.* **55,** 290–296 (2013).
[18] Zhang Hua-Feng., Zhang X., Yangb Xiao-Hua., Qiu Nong-Xue.,Wang Y. et Wang Zhe-Zhi.; *Ind. Crop. Prod.* **50**, 857– 865(2013).
[19] Moreira L., Dias L. G. and Pereira J. A.; *Food Chem. Toxicol.* **46**:3482-3485 (2008).
[20] Carlos Silva J., Rodrigues S., Feás X. and Estevinho L. M.; *Food Chem. Toxicol.* **50**, 1790-1795 (2012).
[21] Ardestani A. and Yazdanparast R.; *Food Chem. Toxicol.* **45**, 2402–2411 (2007).
[22] Omoruyi B.E., Bradley G. and Afolayan A. J.; *BMC Complement Altern. Med.* **12**, 215 (2012).
[23] Sellappan S. and Akoh C. C.; *J. Agri. Food Chem.* **50**, 5338–5342 (2002).
[24] Muanda F. N., Solimani R., Diop B. and Dikco A.; *Food Sci. Technol.* **44**, 1865-1872 (2011).
[25] Duduku K., Rosalam R. and Rajesh N.; *Food Bioprod. Process* **89**, 217-233 (2011).
[26] Oliveira I., Sousa A., Ferreira I., Bento A., Estevinho L. and Pereira J. A.; *Food Chem. Toxicol.* **46**, 2326-2331 (2008).
[27] Mi-Yae S., Tae-Hun K. and Nak-Ju S.; Food Chem. **82**,593-597 (2003).
[28] Kilani-Jaziri S., Bhouri W., Skandrani I., Limem I., Chekir-Ghedira L. and Ghedira K.; S. Afr. J. Bot. 77, 767-776 (2011).
[29] Asadi S., Ahmadiani A., Ali Esmaeili A., Sonboli A., Ansari N. and Khodagholi F.; *Food Chem. Toxicol*. **48**, 1341–1349 (2010).
[30] Wen Xin-Bao., Miao F., Zhou Le., Zhang M. and He Quan-Lei.; *Chin. J. Nat. Med.* **10**(3), 0190-0195 (2001).
[31] Vayalil P.K.; *J. Agric. Food Chem.* **50**, 610-617 (2002).
[32] Hiltunen E., Pakkanen T T. and Alvila L.; *Holzforschung* **60**, 519–527 (2006).

[33] Karabegovi I., Nikolova M., Velickovic D, Stojicevic S., Veljkovic V. and Lazic M.; *Chin. J. Chem. Eng.* **19**(3), 504-511 (2011).
[34] Stojicevic S., Stanisavljevic I., Velickovic D., Veljkovic V. and Lazic M.; *J. Serb. Chem. Soc.* **73** (6), 597-607 (2008).
[35] Vadivel V., Cheong J. N. and Biesalski H. K.; LWT - *Food Sci. Technol*. **47**, 255-260 (2011).
[36] Qing-Feng Z., Yu-Xian G., Xinchen S., Guodong Z. and Wen-Jun W.; *Food Chem.* **133**, 140-145 (2012).
[37] Duenas M., Hernandez T. and Estrella I.; *Food Chem.* **98**, 95-103 (2006).
[38] Jiang F. and Dusting G. J.; *Curr. Vasc. Pharmacol*. **1**, 135-156 (2003).