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Antioxidant proprieties of methanolic and ethanolic extracts of *Euphorbia helioscopia,* (L.) aerial parts

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Abstract: Sun spurge plants (*Euphorbia helioscopia, L.*) were collected from the north of Tunisia. Dried plant parts namely flowers, leaves and stem were individually extracted with methanol and ethanol. Extracts were screened for their antioxidant activity using the 1,1-Diphenyl-2-picrylhydrazyl (DPPH) free radical test. Total phenolics and total flavonoids amounts were also measured. The highest radical scanvenging effect was observed in flowers methanolic extract with IC_{50} value of 26.66 ± 0.000µg/ml. While, relatively poorer antioxidant activity were observed in the same extracts of leaves and stem with respective IC_{50} values of 65.25 ± 0.004 and 80.17± 0.012 µg/ml. The IC_{50} values ranged from 27.55 ± 0.005 to 179.02 ± 0.957µg/ml in ethanol extracts of the above mentioned tested samples. Polyphenols and total flavonoids amounts varied in significant way among tested aerial parts of *Euphorbia helioscopia* and among two used solvents, the highest phenolics and flavonoids contents were found in methanolic flowers extracts (51.49 ± 0.012 mg GAE/g dry weights, 11.38 ± 0.004 mg QE/dry weight respectively). However, ethanol extract of stem gave the lowest amounts of total phenolics and flavonoids (4.80 ± 0.001 mg GAE/g dry weight and 1.69 ± 0.001 mg QE/dry weight respectively).

Keywords: ***Euphorbiacae, sun spurge, total phenolics, flavonoids, DPPH scavenging.***

Introduction

Sun spurge (*Euphorbia helioscopia, L.,* Euphorbiacae family), is an annual plant rising 10 to 50 cm high with erected reddish stem, oval alternate leaves and small yellow green flowers. It is an indigenous plant of North Africa and most of the Europe and Asia. *Euphorbia helioscopia* has been considered as medicinal plant and was used in folk medicine of various countries around the world (Lai *et al.,* 2004; Pieroni *et al.,* 2004; Barla *et al*., 2006; Qureshi *et al.*, 2007). In traditional Tunisian medicine, sun spurge, locally named 'Labeena' (referring to the latex that plant bleeds when cat) have been used as treatment of warts through local applications of the latex on the infected area. A large number of secondary metabolites have been reported from sun spurge plant including diterpenoid, triterpenoid, tannins and steroid (Yamamura *et al.*, 1989; Lee *et al.*, 1990; Wen and Yue-Wei, 2006; Barla *et al*., 2006, Wu *et al*., 2009) which offered to sun spurge herb a wide array of bioactive functions. In fact, *Euphorbia helioscopia* plant was reported to have an antitumor (Cai *et al.*, 1999) a vasodepressor (Barla *et al*., 2006), an antiviral (Ramezani *et al.*, 2008), an antibacterial (Al younes and Abdullah, 2009; Papp, 2004), a nematicidal (Devi and Gupta, 2000), an antifungal (Uzair *et a*l., 2009) and a molluscicidal (AL Zanbagi, 2005) effects. Moreover, *Euphorbia helioscopia* was reported to have antioxidant proprieties (Uzair *et al.*, 2009, Nikolova *et al*., 2011). Nevertheless, in those studies the entire sun spurge plant was investigated but there are no reports about antioxidant activities of separated plant parts. Hence, the objective of this investigation was to evaluate total phenolics, flavonoids contents of methanolic and ethanolic extracts of *Euphorbia helioscopia* leaves, flowers and stem and examine their antioxidant proprieties using the DPPH radical scavenging assay.

Materials and Methods

Chemicals and reagents

All chemicals used in this study were of analytical grade and were purchased from Sigma (USA), Fluka Chemie (Buchs, Switzerland) and Merck (Germany). Deionized distilled water (ddH_2O) was used all through the experiment. All absorbance measurements were

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made using a JSCO V-530 (WITEG Labortechnik., Gmbh) UV-VIS. spectrophotometer.

Plant collection and sample preparation

Sun spurge (Euphorbia helioscopia) plants were collected on April 2008 from the area around the INRAP institute (National Institute of Research and Physical and chemical Analysis) at Sidi Thabet region (latitude 36°55′N, longitude $10^0$04' altitude 41m) which is located at 20 Km North- Ouest of Tunis. This region belongs to the semi arid area of Tunisia characterized by a rainfall range higher than 400mm/ year. Flowers stem and leaves of collected sun spurge plants were separated and dried in the shade at an airy place. Plant materials (flowers, stem and leaves) were then grounded in fine powders and stored in hermetic glass bottles at dark until extraction processing.

Preparation of plant extracts

Powders of stem, flowers and leaves of *Euphorbia helioscopia* plants (100 gram of each plant part) were primarily extracted in Soxhlet extractor by hexane for 6 hours at 65°C in order to remove fatty materials. 25 grams of defatted powders of each plant part material were then individually reextracted in Soxhlet apparatus for 12 hours at 60°C by 250ml of solvent (methanol or ethanol). Following the extractions, solvents were removed using rotary vacuum evaporator at 40°C. Resulting crude extracts were collected into small dark sterile vials and stored at 4°C.

Total phenolics content determination

The total phenolic contents were determined using the Folin-Ciocalteu reagent as described by (Mc Donalds, 2001), 0.5ml of each diluted extract (1:10 mg/ml) was thoroughly mixed with 5 ml of Folin Ciocalteu reagent (1:10 diluted with ddH_2O) and 4ml of (1M) solution of sodium carbonate Na_2CO_3 and allowed to stand for 15 minutes. The absorbance of all samples was measured at 765nm with a UV-visible spectrophotometer. Total phenolics were quantified based on standard curve prepared from various concentrations (0-250 mg/ml) of gallic acid (prepared in 80% methanol) and expressed as mg of gallic acid equivalent per gram of dry weight (mg GAE/g dry weight, standard curve equation: y= 0.005x+0.0173, R2= 0.998). All samples were analyzed in triplicates.

Total flavonoids contents determination

The total flavonoids contents were determined by colorimetric method and expressed as mg quercetin equivalent per g of dry weight, using the method described by Chang *et al*., (2002). 0.5 mL of each plant extracts (prepared from 1mg of crude extract dissolved in 1ml of methanol) were mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride ($AlCl_3$) solution, 0.1 ml of (1M) sodium hydroxide solution (NaOH) and 2.8 ml of ddH_2O. The resulting mixtures were well mixed and incubated for 30 minutes at obscurity. The absorbance of the reaction mixture was measured at 430 nm with a UV/visible spectrophotometer. Samples were analyzed in triplicates. The calibration curve was prepared by quercetin solutions at concentrations of 12.5 µg/ml to100 µg/ml (standard curve equation: y = 0.0078x – 0.0133, R2= 0.999).

DPPH radical scavenging activity assay

The antioxidant activity was investigated using the stable free radical 1,1 diphenyl-2-picrylhydrazyl (DPPH) assay, which estimated the hydrogen donating or the radical scavenging ability of the examined extract. When reacting with an antioxidant compound, the DPPH solution, initially purple in color, changed to yellow. The degree of discoloration denotes the scavenging potency of the tested compound which can be measured spectrophotometrically at 517nm.

The antioxidant activity of Euphorbia helioscopia flowers, leaves and stem extracts was monitored according to the method described by Brand-William *et al.*, (1995) with modifications, briefly 1ml of each sample extract of varying concentrations (20, 50, 100, 200 and 500µg/ml) was mixed with 2ml of DPPH methanol solution (10-4M). The mixture was then allowed to stand at room temperature in the dark for one hour. Absorbance of the mixture was measured at 517nm by an UV-Visible spectrophotometer. Trolox (6-hydroxyl-2578 tertramethylchrome-2-carboxylic acid) was used as positive control and measurements were carried out in triplicate.

Inhibition free radicial DPPH in percentage terms was calculated using the following equation: % of inhibited DPPH= ((AC517-AE517)/ AC517) x100), Where AC517 is the absorbance of the control and AE517 is the absorbance of the plant extract after 1 hour incubation.

Extract concentration that provide 50% inhibition (IC_{50}) and expressed in µg extract /ml was calculated from the graph plotted inhibition percentages against tested samples extracts. The higher the IC_{50}, the lower is the antioxidant activity of the examined sample.

Statistical analysis

Data were analyzed using SPSS statistics 17.0 (release 2008) and expressed as mean ± standard deviation of triplicate determinations. Duncan's test was carried out to determine statistical significance

between the means. Differences were considered statistically significant at the p≤ 0.05 level.

Results and Discussion

Extraction yields

The percentage yields of prepared extracts from the sun spurge plant parts are summarized in table 1. Percentage yields ranged from 4.24% to 19.2%. Furthermore, our results showed that methanol extracts of all three tested parts were found to be higher than those obtained by the ethanol extraction, which is in agreement with several studies reporting the efficiency of methanol in polyphenols recovery compared to ethanol and other solvents of different polarity degrees (Sun *et al*., 2005; Dongmei *et al*., 2007; Anokwuru *et al*., 2011; Chinedu *et al*., 2011; Ghasemzadeh *et al*., 2011). On the contrary, in the investigations of Gayatri and Sahu, (2010) and Koffi *et al*., (2010), ethanol showed better extractive values than methanol and other examined solvents. Alongside the factors that play an important role during the extraction procedure as solvent polarity, extraction time and temperature, sample to solvent ratio (Cacace and Mazza, 2003; Pinelo *et al*., 2005; Chew *et al*., 2011), solvents were reported to have different affinity degrees with polyphenolic compounds which make them more efficient in extracting some phenols better than others, which could partially explain differences in yield extraction among plant parts that originally have different chemical compositions (Niknam and Ebrahimzadeh, 2002; Loganayaki *et al*., 2004; Koffi *et al*., 2010).

Total phenolics and flavonoids contents

As shown in table 1, total phenols expressed as mg gallic acid equivalent/g of dry weight ranged from 4.80 mg GAE/g dry weight to 51.49 mg GAE/g dry weight. Extraction with methanol was found to provide the highest phenolic contents values (13.54, 51.49 and 21.27mg GAE/g dry weight respectively in stem, flowers and leaves) as compared with extraction by ethanol (Table1). Adittionally, Total phenolic contents varied widely among analyzed plant parts, these amounts were significantly higher (P≤ 0.05) in flowers than in stem and leaves in both methanol and ethanol extracts. Likewise, flavonoids contents in samples extracts, expressed as mgQE/g of dry plant weight, differ in significant way (P≤ 0.05) among solvents. While, highest flavoinoids contents were found in methanolic extracts (1.98, 11.38 and 5.32 mgQE/gDW respectively for stem, flowers and leaves), ethanolic extracts showed lower flavonoids amounts (Table 1).

Studies aiming to analyze the amounts of total phenolic compounds in plant organs reported contradictory results; some studies have identified higher amounts of polyphenols in leaves than flowers and remaining plant organs (Basma *et al*., 2011; Chew *et al*., 2011; Govindappa *et al*., 2011; Normala and Suhaimi, 2011). According to Del Bano *et al*., (2004) a number of secondary metabolites are selectively synthesized in leaves, which agree the findings of Chew *et al*., (2011) who noticed rutin and chloragenic acid in leaves but not flowers of both *Bauhinia kockiana* and *Cassia surattensis* plants. However, other studies reported flowers as the richest in polyphenols contents compared to other plant parts, these observations were reported for potatoes (Hyon *et al*., 2008), Ipomoea carnea (Elija *et al*., 2010) and *Justicia spicigera* (Sepulveda-jimènez *et al*., 2009).

Table 1. Total phenolics and flavonoids contents of different extracts of *Euphorbia helioscopia*.

Sample	Solvent	Yield (%)	Total phenolics[a]	Total flavoinoids[b]	IC_{50} [c]
Stem	Methanol	10.64	13.54 ± 0.001	1.98 ± 0.004	80.17± 0.012
	Ethanol	4.24	4.80 ± 0.001	1.69 ± 0.001	179.02±0.957
Flowers	Methanol	19.20	51.49 ± 0.012	11.38 ±0.004	26.66 ± 0.000
	Ethanol	16.52	37.16 ± 0.009	9.26 ± 0.003	27.55 ± 0.005
Leaves	Methanol	13.68	21.27 ± 0.004	5.32 ± 0.002	65.25 ± 0.004
	Ethanol	12.04	14.98 ± 0.007	3.88 ± 0.004	88.49 ±0.002
Trolox	-	-	-	-	11.18 ±0.002

Data are expressed as mean of three measurements ± standard deviation.
(a) mg GAE/g of dry weight; (b) mg QE/g of dry weight, (c) Required concentrations (µg/ml) of the tested extracts to decrease the initial DPPH concentration by 50% .

Polyphenols levels are considerably affected by genetic factors like plant species, plant organ, phenological stage, and environmental factors including both pedo-climatic conditions and biotic and /or abiotic stresses occurring during plant growth (Woodhead, 1981; Conor *et al*., 2002; Fujita *et al*., 2002; Bystricka *et al*., 2010; Stine *et al*., 2011). In the present study, *Euphorbia helioscopia* flowers have showed higher amounts of total phenolics and flavonoids compared to leaves and stem which could be a result of secondary metabolites accumulation, in flower tissues in order to attract pollinators (insects) and protect flowers from biotic and/or abiotic stresses.

Antioxidant capacity

The antioxidant capacity of *Euphorbia helioscopia* methanol and ethanol flowers, stem

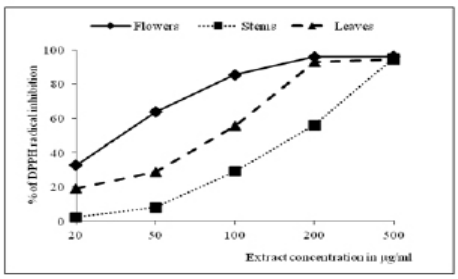

Figure 1. The antioxidant activity of different concentrations of ethanolic extracts of Euphorbia helioscopia plant parts on DPPH radicals after one hour incubation.

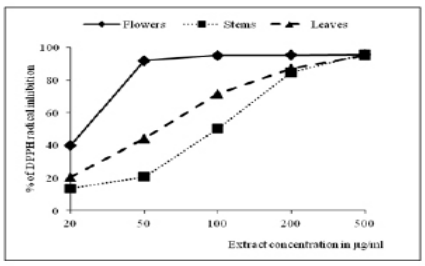

Figure 2. The antioxidant activity of different concentrations of methanolic extracts of Euphorbia helioscopia plant parts on DPPH radicals after one hour incubation.

and leaves extracts was investigated and expressed in terms of IC_{50} which denotes the required sample concentration to inhibit 50% of DPPH in the reaction mixture. Trolox was used as standard. After one hour incubation all extracts showed an antioxidant activity that increased while the concentrations of the analyzed extracts increase (Figs.1 and 2). Nevertheless, methanol extracts showed higher DPPH inhibition percentages compared to ethanol extracts, as an example DPPH inhibition percentages of ethanolic extracts of flowers, leaves and stem at the concentration of 50 µg/ml were respectively 63.55%, 17.41% and 8.04% while, at the same concentration, those of methanolic extracts was respectively 91.92%, 65.96% and 20.70% (Figs.1 and 2). Furthermore, there were significant differences in IC_{50} values among examined samples, the highest ability of DPPH radical inhibition was exhibited by flowers methanolic extract with IC_{50} value of 26.66µg/ml followed by leaves and stem methanolic extracts with respective IC_{50} values of 65.25µg/ml and 80.17µg/ml. while, the lowest antioxidant potency was found in the ethanol extract of stem with IC_{50} of 179.02µg/ml (Table 1). In terms of DPPH radical inhibition and despite used solvent, tested sun spurge parts were classified as follows: flowers > leaves >stem (Figs.1 and 2; Table 1).

The antioxidant activity of *Euphorbia helioscopia* whole plant was previously demonstrated (Nicolova *et al*., 2001; Uzair *et al*., 2009). Likewise, numerous *Euphorbia* species have shown antioxidant proprieties such as *Euphorbia nerifolia*, (Sharma *et al.*, 2011), *Euphorbia tirucalli* (Jyothi *et al*., 2008), *Euphorbia thymifolia* (Chun-Ching *et al*., 2002) and *Euphorbia hirta* (Kandalkar *et al*., 2010). Our data showed that highest antioxidant potency among sun spurge parts was exhibited by flowers, which might be related to high amounts of total phenolics and flavonoids in this plant organ compared to leaves and stem, since phenolics and flavonoids compounds have been recognized as one of the most important groups of secondary metabolites with significant antioxidant and chelating proprieties (Rice-Evans, 1996; Kelly *et al*., 2002; Pietta, 2000; Michalak, 2006). Our data are in agreement with Javanmardi *et al*., (2003), Amatya and tuladhar (2011), Basma *et al*., (2011), Govindappa *et al*., (2011) and Milan *et al*., (2011) who reported the same relationship respectively in case of *Ocimum basilicum* seeds, *Eupatorium odoratum* flowers, *Euphorbia hirta* Leaves, *Wedelia trilobata* leaf and stem and *Teucrium montanum* flowers.

Conclusion

The antioxidant proprieties of *Tunisian Euphorbia helioscopia* flowers, leaves and stem revealed that extracts from flowers had the highest phenolic and flavonoids content as well as the highest antioxidant potency compared to the extracts from leaves and stem, regardless the solvent used. Further investigations regarding more biological activities of the plant parts collected from different geographic locations need to be conducted.

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