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Phytochemical screening, quantitative analysis and antioxidant activity of *Lifago dielsii* Schweinj. & Muschl. (Asteraceae)

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Abstract

This study is designed to assess the phytochemical screening of *Lifago dielsii* Schweinj. & Muschl., endemic species localized in the South of Algeria, and to evaluate their potential antioxidant properties using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging and lipid peroxidation inhibition (LPO; Fe^{2+}/ascorbic acid system) assays. The phytochemical screening of the aerial parts of *L. dielsii* revealed the presence of triterpenoids, saponins, alkaloids, coumarins, flavonoids and tannins. Three fractions [chloroform ($CHCl_3$), ethyl acetate (EtOAc), *n*-butanol (*n*-BuOH)] obtained from aqueous-MeOH extraction and the insoluble methanol (MeOH) part in water, were subjected to a quantitative determination of polyphenols and flavonoids. The antioxidant properties of all extracts were evaluated. The EtOAc fraction had the highest amount of total phenolic contents (TPC) compared to MeOH and *n*-BuOH) fractions whereas $CHCl_3$ fraction showed the lowest level. The *n*-BuOH fraction was richer in total flavonoids content (TFC) (88.81%) compared to EtOAc (37.76%) and MeOH extract (37.88%). The $CHCl_3$ fraction exhibited the weakest content of TFC (18.82%). The antioxidant activity revealed that the EtOAc extract seems to have the most powerful effect on the DPPH scavenging effect (IC_{50}=47.80 ± 2.20 µg/ml) and on LPO inhibition (IC_{50}=113.24±0.65µg/ml). These results showed that *L. dielsii* would be suggested as a promising alternative source of the natural anti-oxidative phenolic compounds.

Keywords: Phytochemical screening, phenolic content, flavonoid content, antioxidant activity, *Lifago dielsii*, Asteraceae.

Introduction

The request for new natural antioxidants has greatly increased, due to the undesirable side effects of synthetic antioxidants and the potential health advantages of natural ones. Many studies have established an inverse relationship between intakes of popular medicinal plants and mortality, from many diseases, which may be attributed to their antioxidant properties [1]. As one of the most important plant antioxidant components, phenolic compounds are widely investigated in many medicinal plants and vegetable. The beneficial effects of phenolic compounds in human life is attributed to their antioxidant activity that mainly due to their redox properties which allow them to act as reducing agents, hydrogen donators [2]. The genus *Lifago* is, a monotypic genus, belonging to the Asteraceae family and representing by only one species: *L. dielsii* Schweinj. & Muschl. This species is an endemic schrub to Westerner Sahara [3]. In South Algeria, the aerial parts of *L. dielsii* are used in folk medicine as antidiabetic. To the best of our knowledge no scientific work on this plant has been performed yet.
As a part of our continuing investigation of medicinal plants of Algeria [4-8], we report here in our preliminary research concerning the phytochemical screening of the aerial parts of this species. Different extracts, $CHCl_3$, EtOAc and *n*-BuOH, as well as insoluble MeOH part in water of the aerial parts of *L. dielsii* were subjected to quantitative determination of polyphenols and flavonoids as well as an evaluation of the antioxidant properties.

Materials and Methods

Plant material

L. dielsii was collected at the flowering stage in April 2011in Southern Algeria and authenticated by Mr. Abdelhakem (Director of forest, Bechar, Algeria) on the basis of Ozenda [3]. A voucher specimen has been deposited, under N LDC /2011, in the Herbarium of the VARENBIOMOL unit research, University of Constantine 1.

Phytochemical screening

Flowers and stems were separated, air-dried and subjected to qualitative tests in order to characterize several chemical groups using standard procedures [9-10].

Preparation of plant extracts

The aerial parts of *L. dielsii* (2000 g) were macerated with MeOH/H_2O (80:20 v/v) for 48 h three times. The crude extract was concentrated at room temp. and diluted with 900 ml H_2O. The remaining aqueous solution was successively extracted with $CHCl_3$, EtOAc and *n*-BuOH.

Determination of total phenolic content (TPC)

The amount of total phenolic in all extracts, ($CHCl_3$, EtOAc and *n*-BuOH) in addition to the insoluble MeOH part in water, was determined with Folin-Ciocalteu's reagent [11]. 5 mg of samples or a standard solution of gallic acid were weighed and dissolved in 5 ml of ethanol. A volume of 0.5 ml of Folin-Ciocalteu's reagent (previously diluted 2 fold with distilled water) was added into test tube containing samples and standard at room temperature for 5 min. 2.5 ml of sodium carbonate (20%) were added and left at room temperature around 90 minutes. The absorbance of mixture was evaluated at 765 nm using a UV-Vis spectrophotometer. The TPC was expressed as gallic acid equivalent (GAE) in mg/g of extract based on the calibration curve.

Determination of total flavonoid content (TFC)

Total flavonoid content was estimated by the aluminum chloride ($AlCl_3$) method [12]. 5 mg of samples or a standard solution of quercetin were weighed and dissolved in 5 ml of methanol. To 2 ml of sample, 2 ml of 2% $AlCl_3$ ethanol solution was added. After one hour, at room temperature, the absorbance was measured at 430 nm. TFC was calculated as quercetin equivalent in mg/g of extract (mgQE/g extract) based on the calibration curve.

Antioxidant activity

DPPH scavenging activity

The free radical scavenging activity of the extracts and ascorbic acid as positive control was determined using the stable radical DPPH [13] with minor modifications. Briefly, 1 ml of various concentrations of the extracts was added to 1 ml of freshly prepared DPPH solution (0.2 mM) in methanol. 60 minutes later, the absorbance was measured at 517 nm. Results were expressed as percentage of inhibition of the DPPH radical according to the following equation:

$$\%\ of\ \boldsymbol{inhibition} = (\boldsymbol{Abs_{CN}} - \boldsymbol{Abs_{ext}})/(\boldsymbol{Abs_{CN}})\ \boldsymbol{x}100.$$

Where $\boldsymbol{Abs_{CN}}$ is the absorbance of the control reaction and $\boldsymbol{Abs_{ext}}$ is the absorbance in presence of the extracts.

Inhibition of LPO induced by Fe^{2+}/ascorbic acid system

The reaction mixture containing rat liver homogenate (0.1 ml, 25% w/v) in Tris-HCl (30 mM), ferrous ammonium sulfate (0.16 mM), ascorbic acid (0.06 mM) and different concentrations of the extract in a final volume of 0.5 ml was incubated for 1h at 37 C and the resulting thiobarbituric reacting substance (TBARS) was measured at 532 nm [14]. Trolox was used as reference. Results were calculated, as percentage of inhibition, according to the following equation:

$$\%\ of\ \boldsymbol{inhibition} = (\boldsymbol{Abs_{CN}} - \boldsymbol{Abs_{ext}})/(\boldsymbol{Abs_{CN}})\ \boldsymbol{x}100.$$

Where $\boldsymbol{Abs_{CN}}$ is the absorbance of the control reaction and $\boldsymbol{Abs_{ext}}$ is the absorbance in presence of the extracts.

Statistical analysis

All assays were estimated in triplicates and results were presented as means ± standard deviation (SD). IC_{50} value which means the effective concentration that proved 50% of activity was calculated for each assay. Statistical comparisons were done with Student's test. Differences were considered to be significant at $p < 0.05$.

Results and discussion

Phytochemical screening

Table1: Phytochemical screening from *L. dielsii* (*Asteraceae*)

Chemical groups	Sample	
	Flowers	Stems
Terpenes	-	-
Sterols	-	-
Triterpenes	+	+
Saponins	+	+
Coumarins	+	+
Flavonoids	+	+
Leucoanthocyans	-	+
Anthocyans	-	-
Quinones	-	-
Tannins	+	+
Alkaloids	+	+

Qualitative phytochemical investigation revealed that flowers and stems contained several phytoconstituents such as triterpenes, saponins, coumarins, tannins, alkaloids and flavonoids. (Table1). These bioactive components are naturally occurring in most plant materials and known to possess interesting biological activity such as antioxidant, anticarcinogenic, antiviral, antibacterial, antidiabetic, anti-inflammatory [15-18].

TPC and TFC

TPC and TFC of all *L. dielsii* extracts are shown in figure 1 The EtOAc extract (112.33 ± 20.01 mgGAE/g extract) was found to have the highest TPC compared to n-BuOH (81.09 ± 19.67 mgGAE/g extract) and MeOH extracts (78.36 ± 9.74 mgGAE/g extract), whereas CHCl3 had showed the lowest level. While the n-BuOH extract (72.02 ± 9.21 mgQE/g extract) was found to have

the highest TFC, compared to EtOAc (42.42 ± 2.17 mgQE/g extract) and MeOH extracts (29.68 ± 4.99 mgQE/g extract). $CHCl_3$ extract had also the lowest TFC (7.98 ± 2.24 mgQE/g extract).

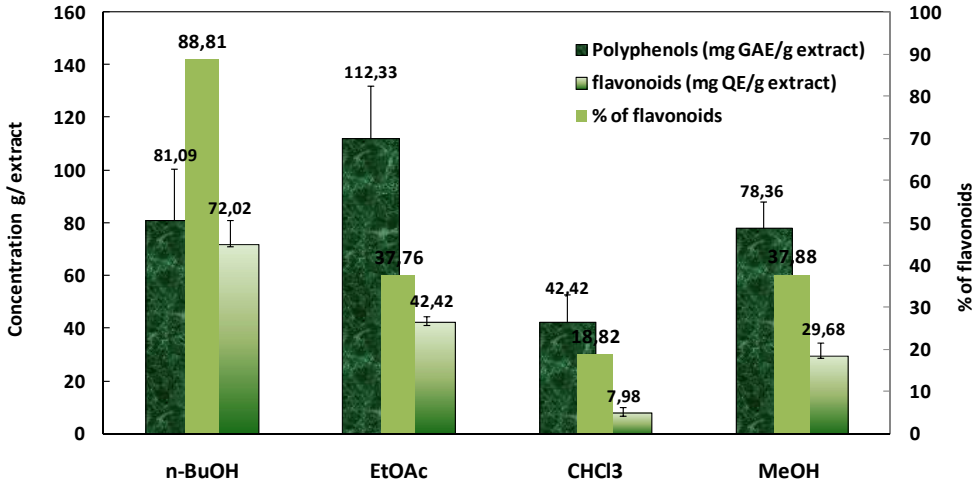

Figure 1: TPC (mgGAE/g extract), TFC (mgQE/g extract), and flavonoids percentage (%) of *L. dielsii* extracts. Each value represents a mean±SD (n=3), $p<0.05$).

The obtained results showed that polyphenols of n-BuOH fraction were richer in flavonoids (88.81 %) than EtOAc (37.76 %) and MeOH (37.88%) fractions. Our results are in good agreement with those obtained by Elgabr & al. (2010) and Öztürk & al. (2009) [19-20]. Polyphenols are broadly distributed in the plant kingdom and are the most abundant secondary metabolites found in plants. The difference in each flavonoid fraction results from the variation in the number and substitution patterns of the hydroxyl groups of these groups. This structural diversity allows them to exhibits many biological activities. The potent antioxidant activity of polyphenols and flavonoids is their ability to scavenge hydroxyl and lipid peroxy radicals. The polarity of the solvent systems used in the extraction could affect the contents of phenolic and flavonoid components [21]. Consequently, the levels of these components that existed in the various solvent extract varied significantly. Our results showed that phenolic and flavonoidic compounds are in greater proportion in polar and semi-polar fractions.

Antioxidant activity

DPPH scavenging activity

In order to evaluate the antioxidant activities of the four extracts of *L. dielsii*, we have used electron transfer based on DPPH scavenging capacity. This assay measure the capacity of an antioxidant to reduce an oxidant that, when reduced, changed color reproducibly. The degree of color change from purple to yellow was correlated with antioxidant potential in extracts.

Figure 2 showed clearly that EtOAc extract was more effective than all the other extracts, and this effect increased until reaching the maximum (78.53 %) at 150 µg/ml. At 200 µg/ml there was no significative difference between EtOAc and *n*-BuOH. The $CHCl_3$ extract (10.66 %) exhibited the weakest effect.

It is also useful to estimate the median inhibitory concentration (IC_{50}), parameter widely used to measure the antioxidant power, and which is defined as the amount of antioxidant concentration needed to reduce the DPPH concentration by the factor of two. Highly effective antioxidant is characterized by low IC_{50} value and vice versa. The IC_{50} values for the four extracts are shown in the figure 4. It can be observed that the ascorbic acid utilized as standard exhibited the highest scavenging effect with 21.37±0.29µg/ml IC_{50} value. The order of increasing IC_{50} for the extracts was EtOAc (47.80 ± 2.20 µg/ml) > n-BuOH (73.31 ± 1.02 µg/ml) >MeOH (512.11± 109.56 µg/ml) (Table 2). Since the polarity of $CHCl_3$ is much weaker than the other solvents, it may contain much non polar components. Many attempts to explain the structure activity relationship of some phenols have been reported in the literature [22]. Halliwell and Guttridge (1999) reported that the power in the anti-oxidation process results first from the ability to prevent the autoxidation of free radical mediates oxidation of the substrates in low concentration and second that the resulting radical after scavenging must be stable.

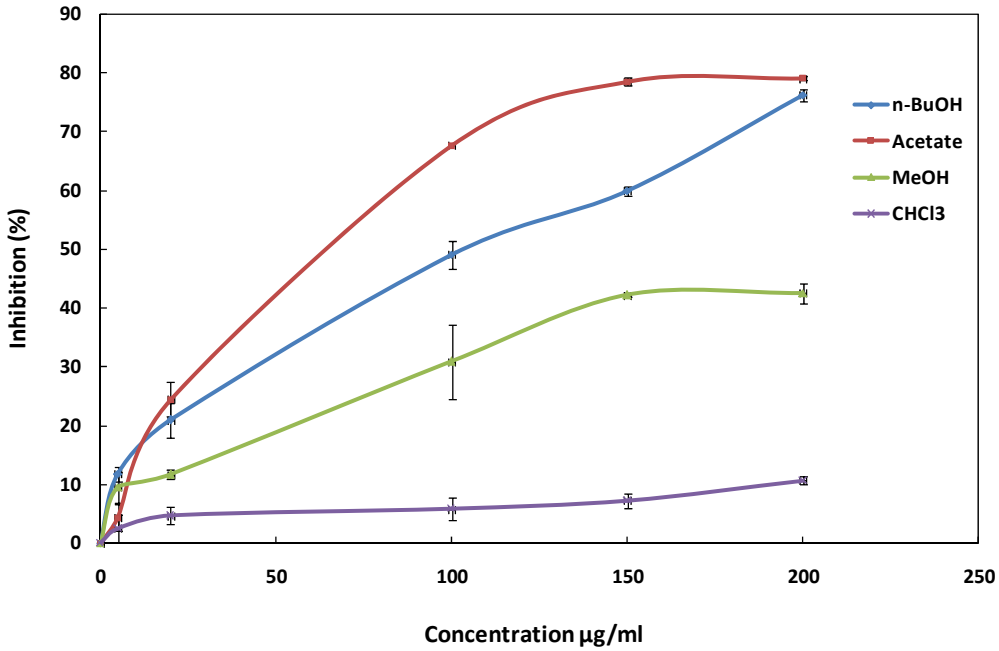

Figure 2: DPPH scavenging activities of *L. dielsii* extracts and standard.Each value represents a mean ± SD (n=3), $p<0.05$

Inhibition of LPO induced by Fe^{2+}/ascorbic acid system

Lipid oxidation occurs when oxygen reacts with lipid in a series of free radical chain reactions that lead to complex chemical changes. Oxidation of lipids not only disturbs the nutritional quality of food, but it is also associated with many diseases [23]. Figure 3 showed that at 200 µg/ml, EtOAc (63.25 ± 0.74 %) possess better inhibition than n-BuOH (54.25 ± 1.55%) and CHCl3 (43.11 ± 0.29%). At 300 µg/ml, EtOAc (80.65 ± 0.59 %) which was better inhibitor of LPO exhibited the same effect as Trolox (81.82 ± 0.29 %).

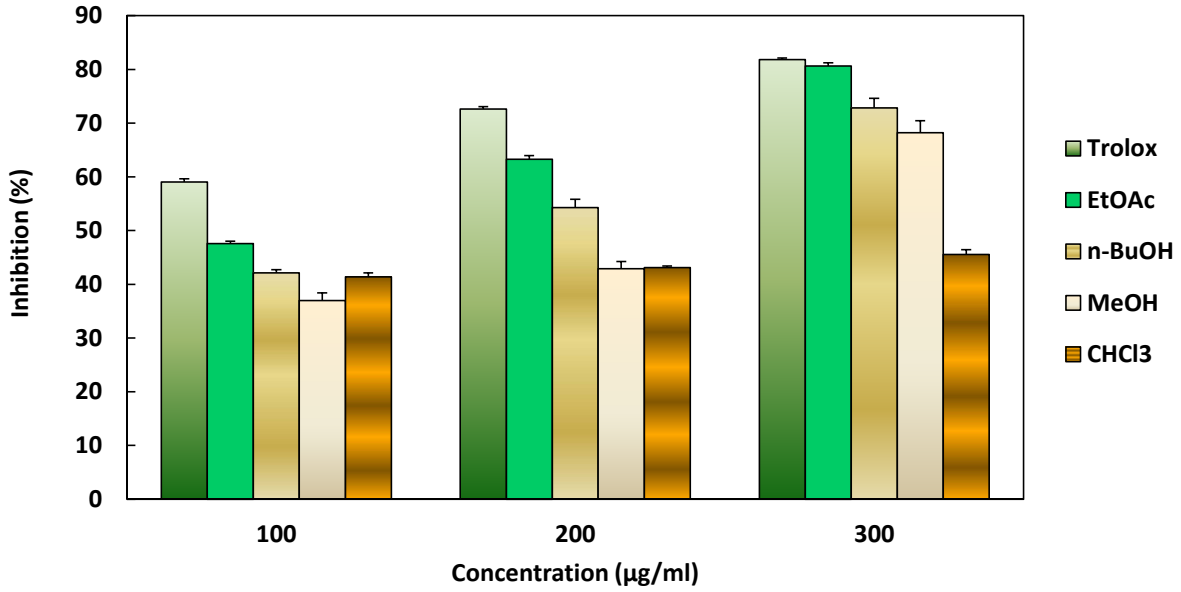

Figure 3: LPO inhibition (%) *L. dielsii* and standard. Each value representsa mean ± SD (n=3), $p<0.05$.

Increased LPO is generally believed to be an important underlying cause of inhibition of antioxidant stress. LPO is regarded as one of the basic mechanism of damage tissue [24]. Our results revealed that EtOAc, *n*-BuOH, and MeOH extracts notably reduced TBARS levels induced in liver homogenates by Fe^{2+}/ascorbate system) with IC_{50} values 113.24 ± 0.65, 143.14 ± 0.77, 184 and 79 ± 6.74 respectively (Table 2).

Table 2: DPPH and LPO IC_{50} values of antioxidant activities of *L. dielsii* and standards (Trolox and Ascorbic Acid). Each value represents a mean ± SD (n=3), $p<0.05$.

Extracts and standards	IC_{50}	
	LPO	DPPH
EtOAc	113.24±0.65	47.80±2.20
n-BuOH	143.14±0.77	73.31±1.02
MeOH	184.79±6.74	512.11±109.56
$CHCl_3$	861.31±77.10	----------
Ascorbic acid	---------	21.37±0.29
Trolox	65.06±3.06	----------

The EtOAc extract which had the highest TPC but lower TFC (37.76 %) exhibited a good LPO inhibition and DPPH scavenging effect. This effect may be explained by the presence of flavonoid type which possesses an ideal structure for decomposing radicals. n-BuOH extract which involves the highest TFC (88.81 %) exhibited more LPO inhibition and DPPH scavenging effect than MeOH although the no difference between their TPC. It is reasonable to conclude that the antioxidant activity is not related only with TPC/TFC level.

Conclusion

The phytochemical screening of this investigation attested the presence of several secondary metabolites in *L. dielsii*. The fractionation of MeOH extract into different phases ($CHCl_3$, EtOAc and *n*-BuOH) solvents resulted into an interesting distribution of TPC and TFC. Some differences in the response of both antioxidant assays to each are noticed. The *n*-BuOH extract provide more antioxidant than MeOH in spite of their comparable content of TPC. The $CHCl_3$ extract exhibited the weakest effect. The EtOAc extract which had the highest TPC was the most powerful in antioxidant activity. The possible synergism of flavonoids with other compounds in the extract may be responsible for these properties. The obtained results indicated that *L. dielsii* would be a promising alternative source of the natural anti-oxidative phenolic compounds. Further research work must be carried out in order to isolate bioactive molecules responsible for their activity.

Author's contribution

MR is supervisor of this study participated in the design conception of this study and involved in drafting the manuscript.
MZ collected the plant and preparing the plant extracts.
AH prepared the plant extracts and phytochemical studies.
AS and MA, have an equal contribution in carried antioxidant activity and statistical analysis, and helping in the drafting the manuscript.
BF and BS have general supervision of the research group.

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