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Assessment of total phenolics and flavonoids, and evaluation of scavenging activity of the aerial parts of *Verbascum thapsus* L. and *Lactuca virosa* L. grown in Algeria

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Abstract

Verbascum thapsus L. and *Lactuca virosa* L. are famous herbs found almost all over the world. They are well-reputed owing to their medicinal properties. They are reported to contain various chemical constituents and are used worldwide for the treatment of various ailments. A number of pharmacological activities regarding their folk use have been documented. Nevertheless there is almost no study concerning the two species in Algeria. Therefore, the present paper aims to contribute to a quantification of total phenolics and flavonoids contents as well as the DPPH scavenging activity of the aerial parts using four extraction solvents: Methanol, chloroform, ethyl acetate and n-butanol. The results revealed that for *Verbascum thapsus*, the n-butanol extract and chloroform extract show the highest total phenolic content (126.35 mg GAE/g) and flavonoids content (83.88 mg QE/g) respectively. Whereas for *Lactuca virosa* it is the ethyl acetate extract that highlights the best content for both phenolics and flavonoids (93.33 mg GAE/g, 49.50 mg QE/g respectively). As far as DPPH scavenging activity is concerned, the results show a good activity of Methanol extract for *Verbascum thapsus* (IC_{50}=0.17), but a moderate activity of ethyl acetate extract for *Lactuca virosa* (IC_{50}=1.02).

Keywords: *Verbascum thapsus*, *Verbascum thapsus*, phenolics, flavonoids, DPPH scavenging activity

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1. Introduction

The use of antioxidants from natural sources as alternatives of the synthetic ones has gained much devotion and efforts in recent years. Moreover the incorporation of local knowledge concerning ethno-botanical relations into biological and ecological studies reinforces the links between man and the environment, leading to the global preservation of biodiversity.

Verbascum thapsus (Great or Common Mullein) is a species of mullein, endemic to Europe, northern Africa and Asia, and brought in the Americas and Australia. It is a bushy biennial plant that can develop up to 2 m or more tall. Its small yellow flowers are compactly grouped on a tall stem, which bolts from a large rosette of leaves. Traditionally, *V. thapsus* has been used to cure headaches, fevers, cramps, burns, and a host of other ailments. The plant contains coumarins and other toxins so it should be used wisely. Moreover, it is reported to find use in the treatment of inflammation, asthma, spasmodic coughs asthma and migraine. Its phenolic constituents are considered to be responsible for the anti-inflammatory and antimicrobial activity of the herb. This biannual plant, flowering from April to May, is also known for its diuretic, analgesic, expectorant and antiseptic properties. The species contains biologically active compounds, such as flavonoids, phenylethanoid and neolignan glycosides, saponins, iridoid and monoterpene glycosides. Although relatively few pharmacological studies on mullein preparations have been reported, the pharmacological activities of certain constituents, notably the iridoid aucubin and the phenylethanoid glycoside verbascoside (acteoside), have been extensively studied [1-4].

V. thapsus anti-bacterial properties make it effective in treating infections. It was applied as remedy against tuberculosis as it inhibits mycobacterium. The leaf extract also revealed in vitro antitumor, antifungal, antiviral, and antibacterial properties. The *V. thapsus* plaster can also

be used for the bruises and to relieve aching and arthritic pains. Mullein flowers oil can be used to cure swollen glands and earaches. Native Americans used the leaves of the plant to alleviate respiratory anxiety. The plant tea is also a useful way of treating respiratory and other ailments such as bronchitis, asthma, and allergies. It is also effective in treatment of pharyngitis and coughs. *V. thapsus* has no serious recorded additional symptoms. However, taking it in surplus dose can result in indigestion and dyspepsia, and it is also careful to lightly brush the thin hairs off the plant leaves as they can result in irritation in some cases [5-6].

Many active compounds were isolated from *V. thapsus* and hence reflecting its biological potency. For instance many studies reported the isolation of iridoid glycosides, iridoids, phenylethyl glycosides, sesquiterpenes, diterpenes, biflavonoids and other minor compounds from the whole plant [7-15].

Wild lettuce (*Lactuca virosa*) is native to several regions worldwide, including North America, Europe and the Middle East. It thrives in sunshiny places, such as along watersides and pavements, and can develop up to six feet (1.8 meters) in height. It has bright green leaves which sprout from a green stem that is sporadically spotted purple. When smashed, the plant secretes a milky, white substance known as lactucarium. When dried, this compound resembles opium, a pain-relieving agent extracted from unripe seedpods of the opium poppy. Lactucarium may deliver similar effects as opium — but with fewer side effects. Historically, wild lettuce was used as a pain reliever and a treatment for conditions such as whooping cough, with research studies on its use dating as far back as 1815. Today, there are many different wild lettuce products available, containing extracts of the plant's seeds, leaves and milky sap. These tinctures, powders, oils and pills are marketed to treat a range of conditions, including anxiety, breathing issues, poor sleep and joint pain [16,17].

Lactucarium has been reported to contain approximately 0.2% lactucin, a sesquiterpinoid lactone. Additionally, the mixture contains a volatile oil, caoutchouc, mannitol, and lactucerol (taraxasterol) (approximately 50%). Lactucerin, also found in the latex, is the acetyl derivative of taraxasterol. Lactucin, lactucopicrin and dihydrolactucin derived from the plant produced analgesic Sedative Anti-cholinergic anti-malarial properties in addition to antifungal and herbicide potential [18-23].

Wild lettuce is as well used for whooping cough, asthma, urinary tract problems, cough, trouble sleeping (insomnia), restlessness, excitability in children, painful menstrual periods, excessive sex drive in women (nymphomania), muscular or joint pains, poor circulation, swollen genitals in men (priapism), and as an opium substitute in cough preparations. The seed oil is used for "hardening of the arteries" (atherosclerosis) and as a substitute for wheat germ oil. Some people apply wild lettuce latex directly to the skin to kill germs. Some people inhale wild lettuce for a recreational "high" or hallucinogenic effect [22-23].

To the best of our knowledge there are poor studies regarding the antioxidant properties of the above plants grown in Algeria, hence the present study is intended to assess the total phenolics content, total flavonoids content as well as the DPPH scavenging activity of different extracts of the two herbs collected in Oum elbouaghi: a semi-arid region east of Algeria.

2. Materials and Methods

2.1 Plant Material

The aerial parts (leaves, flowers and stems) of *Verbascum thapsus* and *Lactuca virosa* were collected from Oum El Bouaghi east of Algeria in July 2018. The plants were identified by Pr. A. Zellagui, Oum El Bouaghi University, Algeria. Voucher specimen VT1 and LV1were deposited in the herbarium of Laboratory of Natural Resources and Management of Sensitive Environments, University of Oum El Bouaghi, Algeria.

The collected plant material was dried in the shade for three weeks before being ground and preserved until use.

2.2 Methanol Extract

200 mg of finely ground plant material were macerated in methanol during 24 h at room temperature. Then the solution was filtered and dried under vacuum and kept at 4°C in the dark until further analysis. After filtration and evaporation the residue is subjected successive extraction with chloroform, ethyl acetate and n-butanol.

2.3 Total Phenolics Content: TPC

The Total phenolics content of each extract was determined using the folin-ciocalteau reagent (FCR) method according to the method of Singleton *et al* [24]. Each extract diluted with methanol (0.5 mL) was added to 2.5 mL of FCR (diluted 1/10 with distilled water) and mixed. After 5 min of agitation, 2 mL of sodium carbonate water solution Na_2CO_3 (75g/L) was added to the mixture and incubated at 40 °C for 30 min. Results were expressed as mg of Gallic acid equivalent (GAE/g of dry extracts). All samples were analyzed in triplicates.

2.4 Total Flavonoids Content: TFC

The total flavonoids content of each extract was estimated according to the colorimetric method using aluminum chloride [25]. This method based on the formation of a complex flavonoid-aluminum having the maximum absorbance at 430 nm. The extracts (1 mL) were mixed with 2% $AlCl_3$ methanol solution (1 mL) and the absorbance at 430 nm was determined using UV-VIS spectrophotometer. The total flavonoids content was expressed as mg quercetin equivalent/g dry extract. All samples were analyzed in triplicates.

2.5 DPPH Radical-Scavenging Activity

The capacity of each sample extract to reduce the radical 2,2-diphenyl-1- picrylhydrazyl (DPPH) was assessed using the method of Masuda *et al.* [26]. 15 µl of each extract at different concentrations was added to 15 µL of a DPPH ethanolic solution. The mixture was shaken vigorously and left standing at room temperature for 30 min in the dark. The absorbance of the resulting solution was then measured at 517 nm. The normal purple color of DPPH will turn into yellow when its singlet electron is paired with a hydrogen atom coming from a potential antioxidant. The scavenging activity of essential oil and methanolic extract was evaluated according to the formula:

$$\textbf{DPPH Scavenging Effect}\ (\%) = \frac{\mathbf{A_0 - A_1}}{\mathbf{A_0}} \times \mathbf{100}$$

where *A*0 is the absorbance of the control at 30 min, and *A*1 is the absorbance of the sample at 30 min. All samples were analyzed in three replications.

2.6 Statistical Analysis

Antioxidant activity, total phenolic content, and flavonoid content are reported as the mean ± standard deviation (SD). Significance differences for multiple comparisons were determined using one way analysis of variance (ANOVA). Duncan's multiple range tests was used to assess the significant differences with the SPSS statistical analysis package (version 15.0; SPSS Inc., Chicago, IL, USA). Differences at $P < 0.05$ were considered statistically significant.

3. Results and Discussions

3.1 Extraction Yield

The yields of methanol crude extracts of the *Verbascum thapsus* and *Lactuca virosa* are found to be 4.74% and 2.72% with respect to dry plant material respectively.

The yields of successive extractions of crude methanol extract using chloroform, ethyl acetate and n-butanol are shown in table 1:

Table 1: different extraction yields of both plants

Extract	*Verbascum thapsus*	*Lactuca virosa*
Chloroform	5.72±0.11	31.40±0.25
Ethyl acetate	7.00±0.17	18.33±0.19
n-Butanol	57.91±0.21	9.12±0.14

3.2 Total Phenolic Contents (TPC)

Phenolics are secondary metabolism components and well established to show antioxidant activity and contribute to human health. The properties of the extracting solvents significantly affected the yields, total amounts of phenolics, and oxidant activity. In this study, the total phenolics content was determined using the Folin-Ciocalteu method, with Gallic acid as a standard. The content of phenolics was evaluated from the regression equation of the calibration curve ($R^2 = 0.9902$, $y = 0.0096\ x$), expressed in milligrams of Gallic acid equivalent per gram of extract (mg GAE/g extract).

Table 2: Total phenolics in different extracts (mg GAE/g)

Plant species	Methanol extract	chloroform extract	ethyl acetate extract	n-butanol extract
Verbascum thapsus	71.15±1.04	63.13±0.52	109.79±0.58	126.35±0.54
Lactuca virosa	44.58±0.92	38.23±0.68	93.33±0.69	74.69±0.62

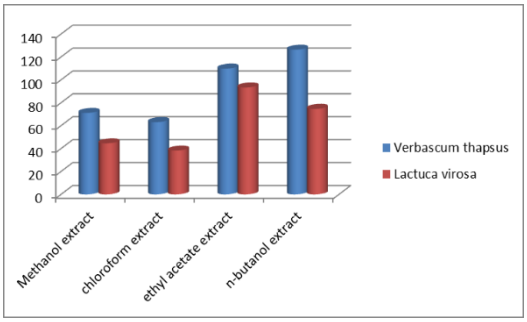


Fig 1: Total phenolics in both plant species

The results exposed important variations in total phenolics amount because of the affinity of phenolics towards different solvents.

3.3 Total Flavonoids Contents

The flavonoids content, expressed in milligram of quercetin Equivalent per gram of dry extract (mg QE /g),

was determined from the regression curve y = 0.0281 x; R^2 = 0.9931 (table 3)

Table 3: Total flavonoids in different extracts (mg QE/g)

Plant species	Methanol extract	chloroform extract	ethyl acetate extract	n-butanol extract
Verbascum thapsus	16.44±0.21	83.88±0.52	83.17±0.37	38.43±0.63
Lactuca virosa	46.37±0.33	48.72±0.31	49.50±0.51	42.42±0.33

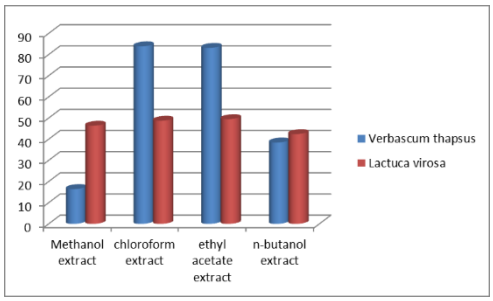
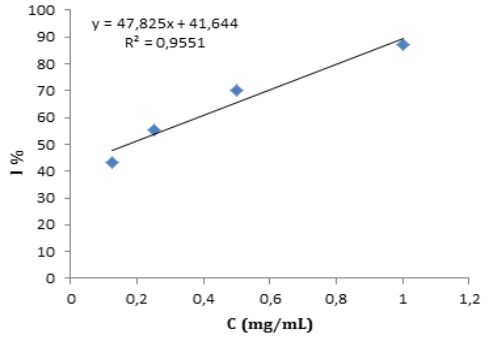


Fig 2: Total flavonoids in both plant species

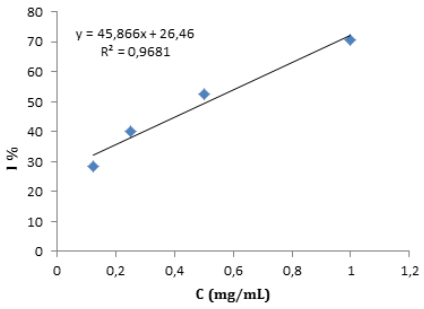
3.4 Antioxidant Activity

Antioxidant activity was determined by free radical-scavenging assay (DPPH). The results are reported in Table 4.

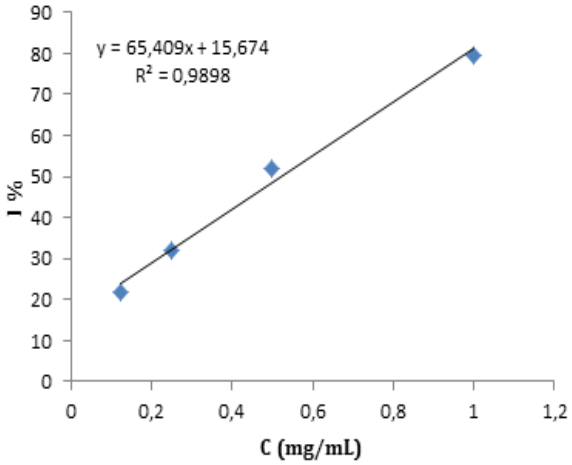
With the DPPH essay, we obtained a stable radical, purple in solution and has a maximum absorption characteristic at 517nm. The routine protocol applied is based on the disappearance of the radical when the DPPH is reduced by a compound with a free-radical property, causing the transformation of the color from purple to yellow. The IC_{50} is defined as the concentration of the sample required to achieve a 50% decrease in the absorbance of the initial solution of DPPH. The IC_{50} values are inversely proportional to the scavenger effect whose low values reflect a significant anti-radical effect. DPPH inhibitory activity of the two species was evaluated and compared with ascorbic acid as a positive control.



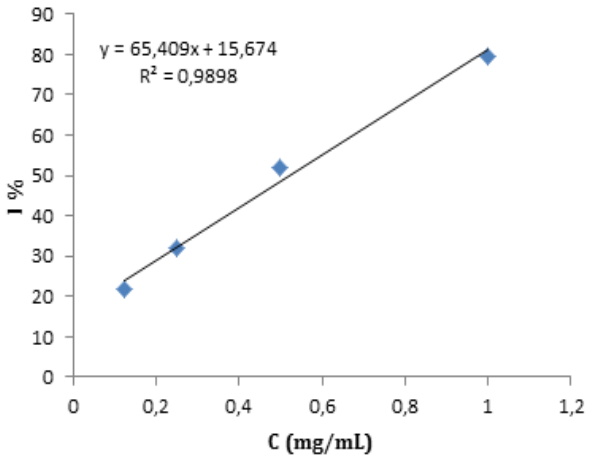
a. scavenging effect of methanol extract



b. scavenging effect of chloroform extract

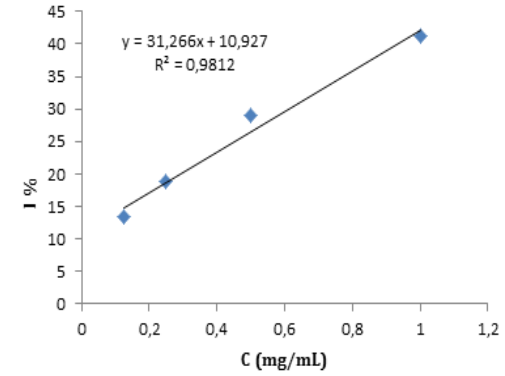


c. scavenging effect of ethyl acetate extract

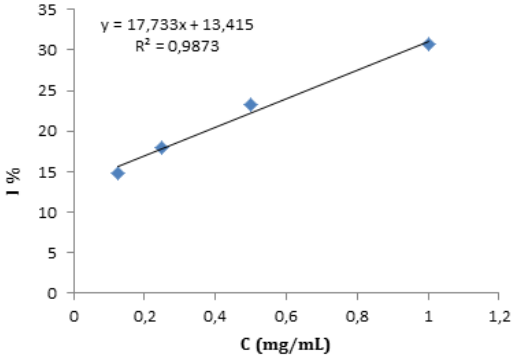


d. scavenging effect of n-butanol extract

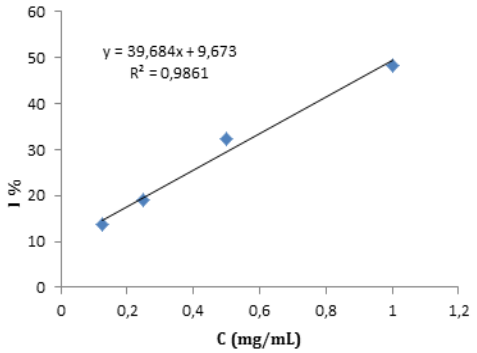
Fig 3. scavenging effect of different extracts of *Verbascum Thapsus*



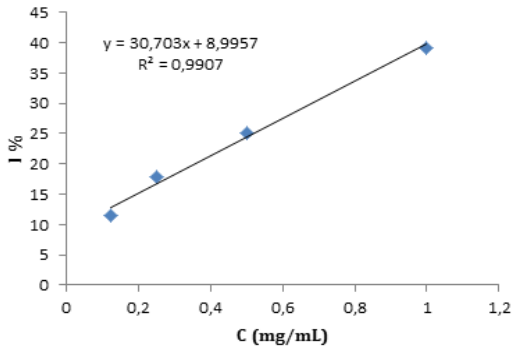
a. scavenging effect of methanol extract



b. scavenging effect of chloroform extract



c. scavenging effect of ethyl acetate extract



d. scavenging effect of n-butanol extract

Fig 4. scavenging effect of different extracts of *Lactuca virosa*

Table 4: IC_{50} values (mg/mL)

Plant species	Methanol extract	chloroform extract	ethyl acetate extract	n-butanol extract	Ascorbic acid
Verbascum thapsus	0.17	0.51	0.52	0.47	0.028
Lactuca virosa	1.25	2.06	1.02	1.33	

Previous studies dealing with total phenolics and DPPH scavenging activity of the above herbs highlighted important fluctuations depending upon the extraction solvent, the area of collection and the phenological stage. For instance, Pal et al (2013) investigated in vitro antioxidant and renoprotective potential of methanolic extract of *Verbascum thapsus* leaf (Nepal india) in rats and found that at a concentration of 0.64 mg/mL of methanol extract, the DPPH inhibition rate is more than 50% which comparable to our results [27]. Another report stated that ethanolic extract of the plant in Pakistan resulted up to 85% inhibition of free radical and upto 40% inhibition by water extract in DPPH assay [28].

Narayanaswamy and Balakrishnan reported that in a DPPH scavenging activity assay with alcoholic and water extracts of *V. thapsus* leaves, the ethanol extract was found to have a higher antioxidative potential than the water extract with an inhibition percentage more than 95% and a total phenolics content around 1mg of catechol equivalent/g of plant [29].

Considering the species *Lactuca virosa*, Anna Stojakowska et al (2012) reported that total antioxidant power (measured as DPPH scavenging activity) of hydroalcoholic extracts from different cultivars of lettuce reached nearly 70% [30]. Though many papers reported investigations on other species of the genius, the one in hand remains relatively poor.

Conclusion

Medicinal plants are important for screening and discovering of the secondary metabolites which are very beneficial for the production of new medicines. The phytochemical analysis of the medicinal plants is so important and has commercial interest in both pharmaceutical and research aspects for treatment of several diseases. The information presented in this study highlights the pharmacological value of *Verbascum thapsus* and *Lactuca virosa* as good natural resources of antioxidants. There is an entire need to identify the phenolics and isolate

new compounds from different parts of the plants, and extend the bioactivity essays for the important activities.

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