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Research Article

FIRST REPORT ON PHYTOCONSTITUENTS, LC-ESI/MS PROFILE AND *IN VITRO* ANTIOXIDANT ACTIVITIES OF *LATHYRUS LATIFOLIUS* GROWING IN ALGERIA

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ABSTRACT. For the first time, this work is devoted to the phytochemical and biological study of a medicinal plant belonging to the Algerian flora Lathyrus latifolius. This research was conducted to assess the phytochemical composition of ethyle acetate ,butanolic and chloroform extracts using LCMS/MS, following by testing in vitro antioxidant ability using DPPH, ABTS·+, O2 − DMSO alkalin, Reducing power, β-Carotene-linoleic acid and CUPRAC assays. The liquid chromatography results showed that ethyle acetate extract have a high amount of Hesperidin (583.31□g/ml) and Quercetin-3-D-xyloside(27.467□□g/ml), while the amounts present in the butanolic extract are respectively (3.360 □g/ml) and (1.812µg/ml). Furthermore, butanol and ethyle acetate extracts had good antioxidant activity in all tests used. Indeed, the presence of phenolic compounds may contribute to their antioxidant activity.

Key words: *Lathyrus latifolius, antioxidant activity, hesperidin*,

INTRODUCTION

Reactive oxygen species have a major role in the development of numerous neurodegenerative diseases like autism, ischemia, Parkinson's syndrome, Alzheimer'sdisease, obesity, diabetes , cancer, cataracts, aging and hepatic disorders [1].

Human cells exhibit various mechanisms that involve free scavenging, metal chelating and enzymatic activities to neutralize free radicals after their development in order to reduce or inhibit the oxidative damage. In addition, supplementation with antioxidants can be helpful in reducing tissue damage caused by oxidative stress when these mechanisms have failed to cope with body need [2].Due to the antioxidant potential of plants[3-5], the phenolics have attracted great attention in recent years[6]. There are two main antioxidants, natural and synthetic, the synthetic ones were found to have long-termtoxicological consequences, including cancer[7].For this reason, there is a growing interest in finding advantageous, less toxic and more effective antioxidants from medicinal species, to remove synthetic antioxidants such as butylatedhydroxytoluene (BHT) and butylatedhydroxyanisole (BHA). In this study one medicinal species was used, *Lathyrus latifolius.* The genus *lathyrus* belongs to the Fabaceae family,many species of this genus are important economically and are used as fodder, food for humans, feed animals, ornamental plants and nitrates to soil [8].In Algeria, *Lathyrus* genus is represented by 22 taxa, this genus is characterized by a calyx with five equal or unequalteeth. Orbicular standard. Stamensdiadelphes or monadelphes, with trans versely truncated tube [9]. The species *Lathyrus* contain flavonoids [10], fattyacids and protein [11]. Despite the importance of *Lathyrus* plants as a source of phenolic compounds and our continued efforts tofind effective and safe antioxidant products [12-13], here we describe for the first time the antioxidant activities of the Algerian *Lathyrus latifolius* using six methods and compared these activities with five antioxidant standards (BHT,BHA,α tocopherol, ascorbic and Tannic acids).

MATERIALS AND METHODS

Plant material

During the flowering period, *Lathyrus latifolius* (Fabaceae) was harvested from Setif, Algeria,in june2021. A specie was identified by Dr Khellaf REBBAS. Species deposited in M'silaUniversity (K. Rebbas, Herbier de labo de Botanique, Univ. de M'sila, N°KR0004).

Extraction of plant

About 200g of aerial parts of *Lathyrus Latifolius* were macerated 3 times in a water-methanol mixture (70/30, v / v) for 24 hours at room temperature. The solution was condensed, evaporating the solvent under vacuum, diluted with 100ml of water, after filtration, the aqueous phase was extracted by a liquid-liquid method, using solvents with increasing polarity, starting with chloroform after ethyl acetate and n-butanol. The organic solutions were concentrated up to 37 ° C under vacuum. Extracts obtained: chloroform extract, ethyl acetate extract, and butanol extract.

Phytochemical Screening

A phytochemical screening of *L.latifolius* was performed to identify the various active chemical constituents present in this spice, such as alkaloids, coumarins, terpenoids, triterpenoids, sterols, anthocyanins, flavonoids, saponins and tannins, using standard phytochemical methods [14-15].

Determination of bioactive Constituents

Total Phenolic Content (TPC)

The total phenolic content of *Lathyrus latifolius* extracts was calculated spectrophthometrically according to the Folin – Ciocalteu method [16] and the findings were expressed as micrograms of gallic acid equivalents per milligrams of extract (µg GAE / mg)

Determination of Total Flavonoid Content (TFC)

The total flavonoid content of the *Lathyrus latifolius* extracts has been calculated using the spectrophthometric method defined by Tel et al [17] and the results were expressed as micrograms equivalent to quercetin per milligram of extract (µg QE / mg).

Liquid Chromatography-Electrospray Ionization-Mass Spectrometry analysis LC-ESI-MS/MS:

The quantification of different phytochemical compounds was achieved by 1260 Infinity II liquid chromatography System (Agilent technology) united with 6460 Triple Quad mass spectrometer and Poroshell 120 EC-C18 (100mm × 4.6mm I.D., 2.7µm) column. The mobile phase consisted of 0.1% formic acid and 5mM ammonium format in water (A mobile phase) and 0.1% formic acid and 5mM ammonium format in methanol (B mobile phase), respectively. The flow rate was 0.4mL/min, the column temperature was maintained at 40°C and the injection volume was 4.0µL.The solvent gradient involved in B mobile phase was follows: 1-12min 15%, 12-30min 50%, 30-32min 90% and 32-35min 10%. An electrospray ionization (ESI) mode was negative and positive operating with nitrogen gasat 300°C wich flow to 11L/min, capillary voltage to 4000V and nebulizer pressure to 15psi.

The ethyl acetate and butanol extracts (2mg/mL). Consequently, the solution was filtered through 0.45µm filters and transferred into vials prior to LC-ESI-MS/MS analysis [18].

Antioxidant activity evaluation

DPPH free radical scavenging test

Radical scavengers were performed according to Blois [19].The findings are compared to the antioxidant standards. The results are reported as inhibition at 50 percent concentration (IC_{50}).

ABTS cation radical test

The capture process of ABTS was performed using Re et al. [20], BHT, BHA, α tocopherol, ascorbic and Tannic acids are used as antioxidants standard. Results are expressed as inhibition at 50% concentration (IC_{50}).

Cupric reducing antioxidant capacity test

The reduction in copper(II) antioxidant capacity was determined using the Apak method [21]. BHT, BHA, α tocopherol, a scorbic and Tannic acids are used as antioxidant standards for activity comparison. Results are reported as A0.50.

Reducing power assay

The performance reduction of *lathyrus latifolius* extracts was calculated according to the Oyaizu method [22]. Results were given as absorbance and compared to BHT, BHA, α tocopherol, ascorbic and Tannic acids. Results were given as A 0.50, referring to the 0.5 absorbance concentration**.**

β-carotene/linoleic acid bleaching assay

The antioxidant activity was assessed using the Marco method of β-carotene-linoleic acid test [23]. BHT, BHA,αtocopherol, ascorbic and Tannic acids are used to compare the operation to antioxidant requirements. Results were given as a concentration of inhibition of 50% (IC_{50}).

Superoxide radical scavenging assay by alkaline DMSO

The scavenging activity of *Lathyrus latifolius* extracts was determined with a slight degree by the alkaline DMSO as described by Madan, [24] Shift optimized for microplate-reader. BHA and BHT, α tocopherol, ascorbic and Tannic acids have been used to compare the operation to antioxidant requirements. The results were given as a concentration of inhibition of 50 percent (IC_{50}).

Statistical analysis

Results of the three measurements are stated as mean value ± SD; linear regression analysis and one-way .ANOVA variance analysis were used to detect important variations ($p < 0.05$) using XLSTAT.

RESULTS AND DISCUSSION

phytochemical screening

Table1 shows that sterols, alkaloids, tannins,flavonoids and terpenoids were present in all extracts. However, saponins were present in ethyl acetate and butanol extracts and were not detected in chloroform extract.

Table 1: Phytochemical constituents present in each extract of *Lathyrus latifolius*

Constituents	Chloroform extract	Ethylacetate extract	Butanol extract
Sterols	+	+	+
Alkaloids	+	+	+
Saponins	-	+	+
Tannins	+	+	+
Flavonoids	+	+	+
Terpenoids	+	+	+

Total phenol content and total flavonoid content

The total phenol contents of *L.latifolius* extracts were quantified. The regression equation of calibration curve of gallic acid was $y = 0.0034x + 0.1044$ ($R^2 = 0.9972$). The results showed that the linear relationship was good in the detection ranges. Ethyl acetate extract had the highest total polyphenol (248.62±3.85 µg GAE /mg extract).The total flavonoid content of *L.latifolius* extracts were measured,the standard,the curve equation of quercetin was $y = 0.0048x$ ($R^2 = 0.997$). Butanolic and ethyl acetate extract had small amount of flavonoids (89.50±3.93 QE/mg extract, 71.17±5.16 QE/mg extract),while chloroform extract contain traces. Our study shows higher values than that conducted by Elena Pastor [25]. Our results are similar to those performed on plants known for their excellent antioxidant activity, such as *Echinacea pallida* , *Echinacea* purpurea, mint and *Hypericum* species [26-28].

Table 2: Total phenol content and total flavonoid content

Extracts	Total polyphenols content (µg GAE/mg extract)	Total flavonoid content (µg QE/mg extract)
CHCl3 extract	153.96±1.54	traces
EtOAc extract	248.62±3.85	71.17±5.16
***n*-BuOH extract**	159.51±5.39	89.50±3.93

Liquid Chromatography-Electrospray Ionization-Mass Spectrometry analysis

A qualitative analysis of constituents present in ethyl acetate and butanolic extracts was performed by LC-ESI/MS. This method was developed, optimized and validated has been applied for the simultaneous determination of 29 phytochemicals as shown in table 3, in the two extracts of *L.latifolius* including 11phenolic acids(Shikimic acid, Gallic acid, Protocatechuic acid, Gentisic acid, 4-Hydroxybenzoic acid, Vanillic acid, Caffeic Acid, Syringic acid, P-coumaric acid, Salicylic Acid, Trans-ferulic acid), 16 flavonoids were identified (Taxifolin, Quercimeritrin, Scutellarin, Cynarin, Hyperocide, Quercetin-3-glucoside, Quercetin-3-D-xyloside, Hesperidine, Kaempferol-3-glucoside, Fisetin, Quercetin, Naringenin, Kaempferol, Tamarixetin, Biochanin A, Diosgenin), one Coumarin and one hydroxybenzaldehyde. Looking at the overall results of the two *L. latifolius* extracts, a high amount of hesperidin (583.31µg/ml),Quercetin-3-D-xyloside (27.46 7 µg/ml), P-coumaric acid (12.07 µg/ml), Salicylic acid (11.945 µg/ml),Vanilic acid (8.011 µg/ml), Syringic acid (7.847 µg/ml) and 4-Hydroxybenzoic acid (6.395 µg/ml).Our results indicate that the chemical composition of the different extracts varies qualitatively and quantitatively depending on the solvent used during the extraction. Therefore, the methods used are complementary and practical to gether. Overall our study is quite close to those found in previous research on *Lathyrus* genus [10-11].

Antioxidant activity

Seven methods were used to determine antioxidant capacity, In our study, the free radical scavenging ability and reducing power results are shown in table4. IC_{50} and $A_{0.5}$ values were compared with antioxidant standards (BHT, BHA, α-Tocopherol, ascrobic and tannic acids). Of all the methods used, ethyl acetate and butanol extracts were the most active. The chloroform extract was least activie. Furthermore, butanol and ethyle acetate extracts were more active than α-Tocopherol in O2− DMSO alkaline assay. Furthermore, butanolic and ethyle acetate extracst showed good antioxidant activity in all methods used. Our findings suggest a correlation between phenolics, flavonoids and antioxidant activities. Little research has been done on the antioxidant properties of *Lathyrus* species. *Lathyrus* from Algeria appears to have the best antioxidant capacity than *Lathyrus* species from Turkey and Spain [25,29]. comparing our study with other studies carried out on *Origuanum* species, widely used in traditional medicine, our results were consistent to those found by ramzan et al[30-31].

Table 3: phenolic compounds in different extracts of *Lathyrus latifolius*

No	Compounds	Ethyl acetate extract (µg \ml)	Butanol extract (µg\ml)	RT (min)	Pre.I(m/z) -> Pro.I (m/z)	Ion polarity	LOD (µg/L)	LOQ (µg/L)	LinearityRange (µg/L	R^2
1	Shikimic acid	ND	1.080	1.17	173.0 -> 93.1	Negative	68.25	210.24	500-8000	0.991
2	Gallic acid	0.492	ND	1.60	169.0 -> 125.0	Negative	4.8	15.25	31.25-1000	0.999
3	Protocatechuic acid	0.544	0.009	2.77	152.9 -> 108.9	Negative	4.62	14.77	31.25-1000	0.997
4	Gentisic acid	0.392	ND	3,10	153.0 -> 109.0	Negative	9.45	32.5	125-2000	0.996
5	4-Hydroxybenzoic acid	6.395	0.168	4.52	137.0 -> 93.1	Negative	19.25	54.12	250-8000	0.999
6	4-Hydroxybenzaldehyde	0.071	0.019	5.72	121.0 -> 92.0	Negative	8.78	26.7	62.5-2000	0.998
7	Vanillic acid	8.011	ND	5.87	167.0 -> 151.8	Negative	22.54	52.1	125-4000	0.999
8	Caffeic Acid	1.531	0.257	5.96	178.9 -> 135.1	Negative	2.63	10.8	31.25-1000	0.999
9	Syringic acid	7.847	ND	6.98	197.1 -> 181.8	Negative	26.98	83.2	250-8000	0.994
10	P-coumaric acid	12.070	1.570	8.47	163.0 -> 119.0	Negative	2.25	7.8	15.625-1000	0.999
11	Salicylic Acid	11.945	0.525	8.72	137.0 -> 93.1	Negative	15.94	47.84	125-4000	0.999
12	Taxifolin	0.565	ND	9.55	304.8 -> 258.9	Positive	39.3	139.2	500-8000	0.998
13	Trans-ferulic acid	1.778	0.279	9.54	193.1 -> 133.9	Negative	12.45	35.32	62.5-4000	0.997
14	Quercimeritrin	0.121	ND	10.68	464.8 -> 302.9	Positive	3.13	10.21	31.25-2000	0.998
15	Coumarin	0.212	0.073	10.56	147.1 -> 91.3	Positive	5.63	15.62	62.5-2000	0.999
16	Scutellarin	0.048	0.122	11.06	462.8 -> 286.8	Positive	2.3	6.2	12.5-800	0.997
17	Cynarin	0.218	ND	11.29	516.8 -> 162.9	Positive	9.39	28.3	62.5-2000	0.994
18	Hyperocide	0.161	0.011	11.76	464.8 -> 302.8	Positive	0.38	2.06	6.25-800	0.998
19	Quercetin-3-glucoside	0.017	ND	11.88	464.8 -> 302.9	Positive	1.04	3.12	12.5-800	0.999
20	Quercetin-3-D-xyloside	27.467	1.812	12.48	432.7 -> 299.5	Negative	45.85	125.8	500-8000	0.999
21	Hesperidin	583.31	3.360	12.47	611.0 -> 302.9	Positive	10.6	38.3	62.5-2000	0.999
22	Kaempferol-3-glucoside	0.027	0.009	13.35	448.8->286.9	Positive	0.61	2.31	6.25-200	0.999
23	Fisetin	ND	0.010	13.56	286.8->137.1	Positive	20.8	68.5	125-4000	0.996
24	Quercetin	0.196	ND	15.04	300.7->150.9	Negative	4.54	12.6	15.625-1000	0.999
25	Naringenin	0.156	0.007	15.15	270.9->119.1	Negative	2.8	7.81	31.25-4000	0.999
26	Kaempferol	0.411	ND	16.97	284.9 -> 116.9	Negative	37.26	128.1	500-8000	0.998
27	Tamarixetin	0.045	0.010	17.51	315.0 -> 299.9	Negative	4.73	15.86	31.25-8000	0.999
28	Biochanin A	0.230	0.287	20.54	284.9 -> 151.9	Positive	2.45	7.81	62.5-2000	0.999
29	Diosgenin	0.025	ND	30.48	415.0 -> 271.0	Positive	3.13	8.19	25-800	0.999

ND: Not detected, Pre.I: Precursor Ions, Pro.I: Product Ions, RT: retention time, LOD and LOQ: limit ofdetection and limit of quantification

Table 4: Antioxidant activities (IC_{50} µg/mL) of *Lathyrus latifolius* extracts

Extracts	DPPH·assay IC50 µg/mL	ABTS+assay IC50 µg/mL	O2− DMSO Alkaline assay IC50 µg/mL	Reducing power assay A0.50 µg/mL	β-Carotenelinoleic acidassay IC50 µg/mL	CUPRAC assay A0.50 µg/mL
Chloroformic extract	141.84±0.66	20.90±0.51	73.00±0.97	249.00±1.00	>200	82.02±0.14
Ethyl acetate extract	83.53±0.26	33.06±0.55	21.56±1.00	234.33±1.15	93.34±0.59	48.17±0.07
Butanolic extract	97.38±0.28	36.71±0.76	20.73±0.29	209.00±0.00	147.29±1.70	59.39±0.38
BHA	6.14±0.41^{c}	1.81±0.10^{c}	>200	7.99±0.87^{d}	0.90±0.02^{e}	6.62±0.05^{e}
BHT	12.99±0.41^{b}	1.29±0.30^{c}	>200	>200	1.05±0.01^{e}	8.97±3.94^{e}
α-Tocopherol	13.02±5,17^{b}	7.59±0.53^{b}	31.52±2.22^{a}	34.93±2.38^{b}	1.79±0.03^{e}	19.92±1.46^{e}
Ascrobic acid	13.94±2.81^{b}	1.74±0.10^{c}	7.59±1.16^{d}	6.37±0.42^{d}	52.59±1.98^{b}	12.43±0.09^{d}
Tannic acid	7.74±0.19^{c}	1.01±0.16^{c}	0.94±0.22^{e}	41.07±2.36^{a}	7.46±0.26^{d}	3.76±0.73^{f}

IC_{50} and $A_{0.50}$values is defined as the concentration of 50% inhibition percentages and the concentration at 0.50 absorbance respectively. IC_{50} and $A_{0.50}$ were calculated by linear regression analysis and expressed as Mean±SD (n=3). The values with different superscripts (a, b, c,d or f) inthe same columns are significantly different ($p < 0.05$)

CONCLUSION

According to some studies, some plant species on earth possess therapeutic value, and many medicinal plants have high antioxidant capacity. The butanol and ethyle acetate extracts of *Lathyrus latifolius* have good antioxidant activity due to the presence of flavonoids such as hesperidin, Quercimeritrin, Cynarin as well as phenolic acids like Gallic and Ferulic acids. Furthermore,this antioxidant capacity is due to synergy effect between the components present in extracts.

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