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DETERMINATION OF PHENOLIC COMPOUNDS AND ANTIOXIDANT ACTIVITY OF WHITE HENBANE (*HYOSCYAMUS ALBUS* L.) PLANT TREATED BY KINETIN (K)AND 2,4-DICHLOROPHENOXYACETIC ACID (2,4-D) IN MILA, ALGERIA

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ABSTRACT

The present work deals with the evaluation of the antioxidant activity of the extracts of the white henbane (Hyoscyamus *albus* L.) of the solanaceae family, treated with phytohormones 2,4-Dichlorophenoxyacetic acid (2, 4-D) andKinetin (K)in doses 0, 10,20mg / l), widely used in traditional medicine and very rich in alkaloids. Preliminary phytochemical screening showed that treatment with phytohormones had no effect on the quality of the secondary metabolites. The results of the phytochemical study of the plant showed that the treatment with 2,4-D and K had a significant effect by reduction compared to the untreated plant on the phenolic compounds studied. The evaluation of the antioxidant activity *in vitro* was investigated by the DPPH trapping method and showed that the treatment with 2,4-D and K had a significant effect on the antioxidant activity so all the extracts of the various treatments of *H. albus* can act as radical scavengers.

Keywords: *Hyoscyamus albus* L.; phenolic compounds; antioxidant activity; K; 2, 4-D.

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1. INTRODUCTION

Nature is a unique source of structures of high Natural substances diversity. Plants produce a wide range of secondary metabolites [1]. The medicinal properties of plants are attributed to the presence of secondary metabolites such as terpenoids, steroids, saponins, tannins, flavonoids, alkaloids and phenolic compounds [2]. These substances have taken advantage of multiple interests in the biotechnology industries both in food, cosmetic and pharmaceutical. Between these among are found much of secondary metabolites which are mainly used in therapeutics. Traditional medicines has used for long time plant materials for healing without knowing what had caused their actions, then studies of these substances are the subject of numerous *in vivo* and *in vitro* studies[3]. These include phenolic compounds, polyphenols, flavonoids, tannins which are widely used in therapeutics as vasculoprotective, anti-inflammatory, enzyme inhibitors, antioxidant and anti free radical. The goal of our work is to determinate phenolic compounds and evaluate the antioxidant activity of *Hyoscyamus albus* L. (white henbane) from solanaceae family treated by tow phytohormones: auxin by 2,4-Dichlorophenoxyacetic acid (2,4-D) and cytokinins by Kinetin (K) isolated and combined in doses 0, 10 and 20 mg/l to enhance secondary metabolites. White henbane is an annual and perennial plant [4]traditionally used to treat many diseases in north of Africa; it affect the parasympathetic nervous system and ophthalmology, an aesthesia and treatment of cardiac and gastrointestinal diseases [5].

2. EXPERIMENTAL

2.1 Collection of plant material

Seeds of white henbane were collected from Wilaya of MILA situated in East of Algeria in Jun. They were germinated under controle conditions (shelter) in pot containing soil and peat (5:1 ratio) [6]in university of LARBI BEN M'HIDI, at Wilaya of Oum El Bouaghi East of Algeria (coordinates: 35° 52' 39'' N. longitude: 7° 6' 49'' E). Phytohormones (2,4-D and K) were applied in April at three doses 0, 10 and 20mg/l isolated and interacted (auxin X cytokinin) in three factorial randomized complete block designed with three replications. In the month of June shoots samples (fig. 1) were hand harvested and were dried 15 days in the

shade on air and under ambient temperature until total dehydration. Finally they were powdered finely ground to be used for phytochemical and antioxidant analysis.

Fig.1. *Hyoscyamus albus* L. under controle conditions (aerial part)

2.2 Preparation of Methanol extracts

Ten gram of powder of aerial part of different treatments were extracted by 100ml of Methanol (70%) by maceration for 48 h. The extracts were filtered using WATMAN filter paper (N°1) and then concentrated at 40°C using a vacuum Rotary evaporator. Extracts were stored in freezer for analysis.

2.3 Preliminary phytochemical analysis

Qualitative phytochemical tests for the identification of bioactive components were carried out for all the extracts by the method described by Harborne[7], Sazada *et al.*[8]and Shanmugam *et al*. [9].

2.4 Determination of total phenolic compounds, total flavonoids and tannins

2.4.1 Determination of total phenolic compounds

The phenolic contents were given with the method of Folin-Ciocalteu [10]. Each extract diluted ten times was left; react with 1 ml of the reagent of Folin-Ciocalteu, and then the mixture was neutralized with Na_2CO_3. The mixture was after incubated at ambient temperature during 90 min. Finaly; absorbance was measured with the spectrophotometer assistance at 750 *nm*. T with total polyphenols in our extracts, was calculated starting from a linear calibration curve (y=ax+b*)* established with precise concentrations of gallic acid (0-120

µg/ml*)*, like standard of reference, under the same conditions as the sample. The total phenolic contents were expressed in µg equivalent of gallic acid (GAE)/mg of sample (Fig. 2).

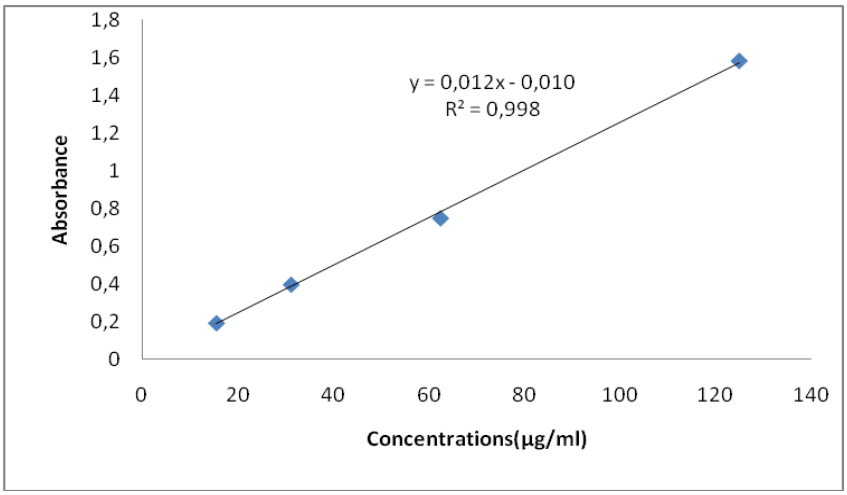

Fig.2. Gallic acid calibration curve

2.4.2 Determination of total flavonoids compounds

The total flavonoïds were estimated by using the method of Ordonez et *al.*[11]. 0.5 ml of of $AlCl_3$ of 2 % (ethanolic solution) were added to 0.5 ml of each extract. After 1 h of incubation at ambient temperature, the absorbance is measured at 420 nm. Content of flavonoïds is expressed in microgram equivalent of quercetin per milligram of extract (µg Eq AQ/mg) starting from the calibration curve established by quercetin (0-25 µg/ml *)*(Fig. 3).

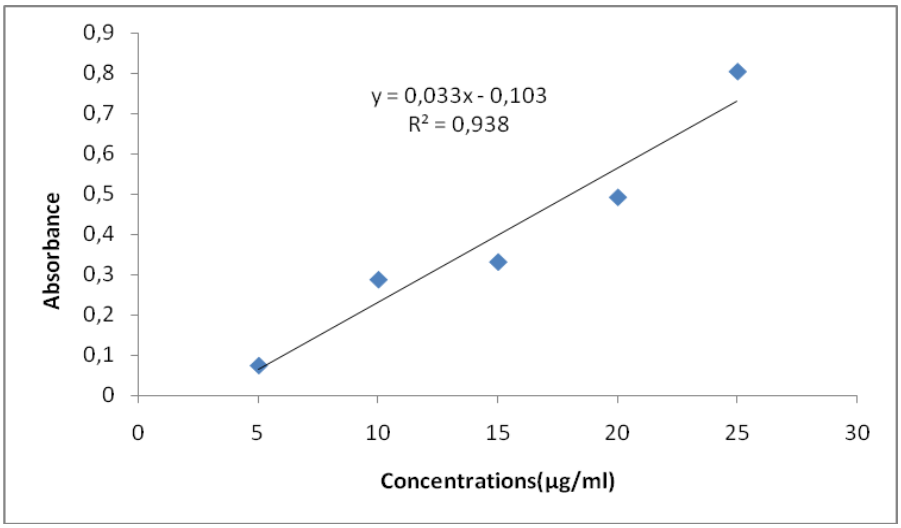

Fig.3. Standard curve for Quercetin

2.4.3 Determination of condensed tannins compounds

The proportioning of condensed tannins in extracts of *H.albus* treated with phytohormones is carried out according to the method of Heimler et *al.*[12]. For 400µl of extract of each treatment, we add 3 ml of a vanillin solution (4% in methanol), and 1.5 ml of concentrated HCl. The mixture is incubated during 15 min and the absorbance is read with 500 nm. Concentrations of the condensed tannins are deduced starting from the ranges of calibration established with the catechin (0-10 µg/ml) and are expressed in microgram of catechin equivalent per milligram of extract (µg ECT/mg) (Fig. 4)

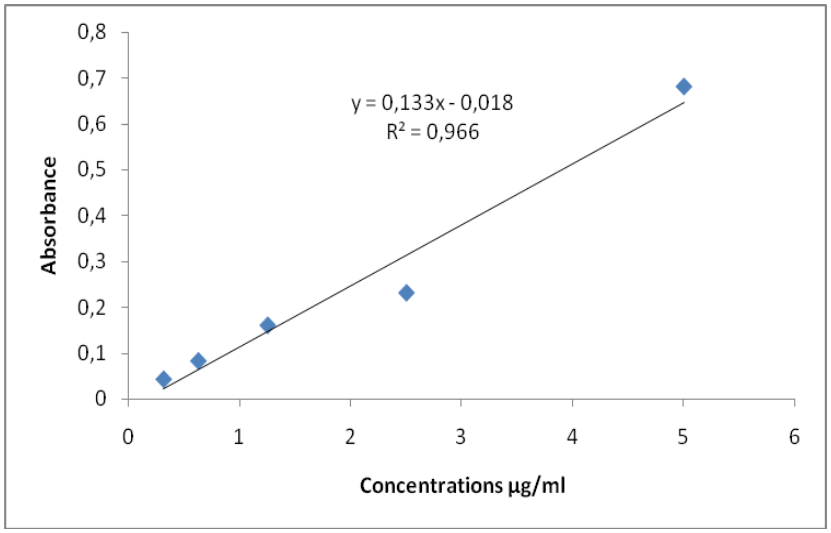

Fig.4. Standard curve for Catechin

2.5 Antioxidant activity using the free radical scavenging activity (DPPH)

The method described by Tepe *et al.* [13] was used with slight modification, the different extracts and controls (Ascorbic acid "antioxidants reference"). DPPH solution was prepared by dissolving 3 mg of DPPH in 100 ml of methanol. Samples and controls were added to 2 ml of DPPH solution after incubation for 30 min, absorbances were measured at 517 nm against the blank. All the assays were carried out in triplicates with Ascorbic Acid as a positive control. Percentage of inhibition (DPPH scavenging activity) determined as follows:

$$\% \text{ Antioxidant activity} = [(\text{Abs control} - \text{Abs sample}) / \text{Abs control}] \times 100$$

2.6 Statistical study

Data obtained were presented as (mean ± standard) deviation of three independent

determinations. All results were subjected to statistical analysis of variance (ANOVA) and correlation using MINITAB (version15) packadge at p<0,01 significant level.

3. RESULTS AND DISCUSSION

3.1 Results of preliminary phytochemical analysis of extracts from different treatments of white henbane

The phytochemical screening of white henbane extracts was done to demonstrate the presence of different types of phytocompounds like Alkaloids, Saponins, Flavonoids, Steroids, Tannins, etc. which could be responsible for the various pharmacological properties [14]. The results reported in table 1 showed positive result with presence of Alkaloids, flavonoids, steroids, saponins, phenols, terpenoids, tanins in the methanolic extracts of all treatments of aerial part of White henbane how gives their therapeutic and medicinal properties [15]. Same result was founded by Benhouda *et al.* [16] how had reported that methanolic extract of *H. albus* recolted from Wilaya of Batna (East of Algeria) contained terpenoids, alkaloids, tannins, flavonoids, polyphénols.

Table 1. Results of preliminary phytochemical analysis of extracts from *H. albus*

	Secondary metabolites						
Treatments	**Terpenoids**	**Alkaloids**	**Saponins**	**Steroids**	**Phenols**	**Flavonoids**	**Tanins**
untreated	+++	+++	++	++	++	+	+
K(10mg/l)	+++	+++	++	++	++	+	+
K(20mg/l)	+++	+++	++	++	++	+	+
2,4-D(10mg/l)	+++	+++	++	++	++	+	+
2,4-D(20mg/l)	+++	+++	++	++	++	+	+
Kx2,4-D(10x10mg/l)	+++	+++	++	++	++	+	+
Kx2,4-D(10x20mg/l)	+++	+++	++	++	++	+	+
Kx2,4-D(20x10mg/l)	+++	+++	++	++	++	+	+
Kx2,4-D(20x20mg/l)	+++	+++	++	++	++	+	+

+++: high ++: moderate +:mild

3.2 Quantitative analysis of polyphenols, flavonoids and tannins compound

Table 2 showed the total phenolic content, flavonoids content and tannins content founded in the extracts of aerial part of *H.albus* treated by 2,4-D and K with doses of (0, 10 and 20 mg/l).

Table 2. Determination of polyphénols, flavonoids and tannins compound

Treatment*	Polyphenols**(µgEGA/mg E)***	Flavonoids**(µgEQ/mg E)***	Tannins**(µgECT/mg E)***
untreated	**354.26±1.36a**	**163.65±1.73a**	**100±1a**
K(10mg/l)	161.03±0.66d	12.29±0.43i	**65.37±0.48b**
K(20mg/l)	169.26±0.47c	45.14±0.69e	18.13±0.58d
2,4-D(10mg/l)	**186.45±0.52b**	116.45±0.65b	20.66±0.67c
2,4-D(20mg/l)	24.12±0.64i	21.76±0.53f	14.33±0.5f
Kx2,4-D(10x10mg/l)	26±0.51h	15.99±0.65g	15.54±0.3f
Kx2,4-D(10x20mg/l)	42.37±0.54f	13.9±0.88h	16.18±0.18f
Kx2,4-D(20x10mg/l)	36.50±0.6g	**128.7±0.47b**	21.16±0.04c
Kx2,4-D(20x20mg/l)	39.44±0.6g	73.03±0.8d	17.32±0.6d

* Values are shown as mean and standard deviation (n = 3);

letters (a–g) Different letters share significant differences at $p < 0.05$.

All the methanolic extracts of aerial part of *H. albus* treated by phytohormones were contained considerable amounts of phenolic compounds and significant differences were observed between the treatments.

The untreated white henbane plant showed the higher amount of total phenols with (354.26±1.36) µgEGA/mg E, of flavonoids with (163.65±1.73) µgEQ/mg E and the higher amount of tannins with (100±1) µgECT/mg E as compared to the all treatment that had showed a less amount of phenolic compounds.

Content of polyphenols of methanolic extract of aerial part of *H. albus* treated by phytohormones ranged from (186.45±0.52) µgEGA/mg E by treatment with 2,4-D(10mg/l) to (24.12±0.64) µgEGA/mg E by treatment with 2,4-D(20mg/l).

Content of flavonoids of methanolic extract of aerial part of *H. albus* treated by phytohormones ranged from (128.7±0.47)µgEQ/mg E by treatment with Kx2,4-D(20x10mg/l) to (12.29±0.43) µgEQ/mg E by treatment with K(10mg/l).

Content of tanins of methanolic extract of aerial part of *H. albus* treated by phytohormones ranged from (65.37±0.48) µgECT/mg E by treatment K(10mg/l)to (14.33±0.5) µgECT/mg E by treatment with 2,4-D (20mg/l).

Benhouda *et al.* [16] had reported that the content of leaves of *H. albus* recolted from Wilaya of Batna (EAST of Algeria) of polyphénols, flavonoids and tannins were (111.1 ± 1.82) µgEGA/mg E, (24.31 ± 0.62) µgEQ/mg E and (24.87 ± 1.57) µgECT/mg E respectively.

Our results indicate that *H. albus* is a good source of phenolics compounds and the used phytohormones had a decrease effect on phenolic compounds accumulation which depends on several factors such as temperature, UV-light, nutrition available to the plant, and genetic factors [17].

3.3 Evaluation of antioxidant activity

Table 3 showed percentage of Radical Scavenging Activity determined by DPPH method of extracts of aerial part of *H. albus* treated by phytohormones that showed a significant difference between all treatments and all % Radical Scavenging Activity (RSA).

Table 3. Percentage of Radical Scavenging Activity (% RSA) extracts of aerial part of different treatments of *H. albus*

Treatments*	**% RSA***
Untreated	88±1b
K(10mg/l)	87±1b
K(20mg/l)	66±1c
2,4-D(10mg/l)	14.33±0.37g
2,4-D(20mg/l)	56.36±1.8e
Kx2,4-D(10x10mg/l)	31.06±0.64f
Kx2,4-D(10x20mg/l)	88±1b
Kx2,4-D(20x10mg/l)	13.26±0.68g
Kx2,4-D(20x20mg/l)	62.46±0.5d
Ascorbic acid	100±1a

* Values are shown as mean and standard deviation (n = 3);

letters (a–g) Different letters share significant differences at $p < 0.01$.

The examination of percentage of RSA of plant extracts from *H. albus* treated by phytohormones showed different values. The obtained values varied from 13.26±0.68% for treatment with Kx2,4-D(20x10mg/l)to 88±1% for both of treatment with Kx2,4-D(10x20mg/l)

and untreated plant which presented percentage near to percentage of the used reference standard (Ascorbic Acid). This result is higher than what was founded in leaves of Libyan H. albus spiece that gives an antioxidant activity of (60.4± 1.1) [18].

Based on results of this study, the untreated plant extract had given the highest antioxidant activity (%RSA) and the highest concentration of phenols (polyphénols, flavonoids, and tannins).

Phenols are very important plant constituents because of their scavenging ability on free radicals due to their hydroxyl groups.

They had reported that The biological functions of flavanoids apart from its antioxidant properties include protection against allergies, inflammation, free radicals, platelet aggregation, microbes, ulcers, hepatoxins, viruses and tumors[19] [20] [21].

3.4 Correlation of measured variables

Table 4. Correlation of measured variables

	Polyphenols	Flavonoids	Tannins	%RSA
Polyphenols	1,000			
Flavonoids	0,583*	1,000		
Tannins	**0,831****	0,448*	1,000	
%RSA	0,345	-0,281	0,523*	1,000

*: Significant **:Highly significant

The results of relationships showed that tannins were correlated positively and significantly with polyphenols (R^2=0,831)and flavonoids were positively correlated with polyphenols (R^2=0,583). A significant relationship between % RSA and tannins (R^2=0,523) who means that the percentage of Radical Scavenging Activity was due to tannins activity in this spice treated by K and 2,4-D. This result justifies their usage in traditional medicines. It is suggested that the polar molecules present in the vegetable extracts contribute to the increase in the antiradical activity [22, 23].

4. CONCLUSION

Phenolic compounds are molecules widespread in the plant kingdom. These compounds are produced in the secondary metabolism of fruits, herbs, and vegetables.

Based on our results, it is possible to conclude that the used phytohormones (2,4-D and k with doses of 0.10 and 20mg/l separed and interacted) had a negative significant effect on phenolic compounds accumulation in white henbane that presented a good source of this one. The high phenolic compounds exhibited the good antioxidant activity in the *H. albus* extracts. It noticed that treatment with 2,4-D and K decreased phenolic compounds in white henbane. We can reveal that *H. albus* presented a rich source of antioxidant compounds. The antioxidant effect of the *H.* albus extracts can also have a biological interest because it can prevent the oxidation of lipidic component in cellular membranes and consequently vegetable exract can appear beneficial for health.

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