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Phytochemical Analysis, Antioxidant and in vivo Biological Activities of Methanolic and Aqueous Extracts of *Datura stramonium*

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ABSTRACT

Datura stramonium is an annual herbaceous plant of the Solanaceae family. The main objective of this study was to evaluate the phytochemical composition, antioxidant, antifungal and insecticidal activities of aqueous and methanolic extracts. Chemical tests and spectrophotometric methods were used for antioxidant activity. The *in vitro* and *in vivo* antifungal potential against *Fusarium* was assessed by the filter and blotting paper technique, respectively. The insecticidal potential was evaluated by repellency test on *Tribolium castaneum*. Data showed that the both of the methanolic and aqueous extracts are rich in phenolic compounds. Antioxidant activity showed that the organic leaves extract has the most important antioxidant activity. For the antifungal activity, we noted that aqueous extracts of roots inhibited mycelial growth of fungi. The aqueous extracts by a decoction of the leaves caused 57.5% repulsion. This data demonstrate *Datura stramonium* may be a new alternative for the development of biological fungicides and insecticides.

Keywords: *Datura stramonium*, aqueous extract, organic extract, antioxidant activity, antifungal activity, insecticidal activity.

INTRODUCTION

Plants perform as a renewable natural resource of diverse bioactive compounds [1]. Natural products have been exploited by humans for thousands of years and used as foods, drugs, antioxidants, flavors, fragrances, dyes and insecticides. Thus, natural compounds have increasingly become the focus of those interested in discovery of pesticides. In fact, Harvest grains are basic human food products thus, the presence of pests leads to a serious problem in grain stocking and its received from industry [2,3]**.** Worldwide, between 5% and 10% of the total weight of cereals, oil plants, and (peas, beans, lentils, etc.) are lost due to the presence of pests [4]**.** Among these pests, the red flour hard-shelled insect, *Tribolium castaneum* (Herbst) is one of the most important pests disgusting stored grain which is widely spread and very destructive. Due to this high rate of infestation, chemical insecticides have been used to control it, but resistance and poisonous quality problems derived from synthetic insecticides have made it necessary to find more effective and healthier alternatives [5].

Additionally, plant diseases are caused by pathogens such as fungi, bacteria, nematodes and viruses. Compared to other plant parasites, fungi cause the greatest impact relating to diseases and crop production losses. This includes relatively large green plants and post-harvest losses of fruits and vegetables which are brought about by rotted due to fungal plant pathogens. Some famous plant disease-causing fungi include Pythium, Phytophthora, Fusarium and Rhizoctonia spp, which cause root and crown rot, seedling damping-off in many vegetables and ornamental plants [6]. To overcome these diseases, synthetic fungicides have been used worldwide for enhance crop production and control plant disease. Unfortunately, when fungi are treated with chemical fungicides over a long period, some of them develop resistance to these fungicides. These chemicals are also imposing adverse effect on other non-target microbes and disturb the microbial community of soil [7]**.** View of these facts, it is an urgent need to discover ecofriendly, renewable non-phytotoxic, relatively cost effective and biodegradable botanicals fungicides. Thus, the use of plant-derived bioactive compounds which are effective, accessible, safe, and less expensive could be an important treatment option for the development of novel biological pesticides. In fact, research in the field of plant-based pesticides offers a very promising ground in the wake of new concepts of development of sustainable agriculture and the protection of natural resources especially that the Tunisian territory is rich in plants that can exploit them. *Datura stramonium*, a widespread annual plant belonging to *Solanaceae* family would be a candidate for such assays.

The *Solanaceae* family is one of the most used as a food plants and provide fruits, spices, and stimulants, as well as chemical compounds which are important in medicine, pharmacology, and drug therapy [8]. It is widespread in tropical and subtropical regions of the entire world [9]. It is considered as the third most economically important families in plant kingdom after the Poaceae and Fabaceae [10].

Within this family *Datura stramonium* have received particular consideration, thanks to its chemical composition and its various medicinal and pharmacological properties [11]. This plant is grown in temperate and tropical region of the globe. In Tunisia, thorn apple is easily cultivated, growing well in open,

sunny situation. It flourishes in most moderately good soil but grows best in calcareous rich soil, or in a good sandy loam, with leaf mould added [12]. Some studies have also stated their antioxidant and antimicrobial properties, but most of them dealt with organic extracts; whereas only a few studies report the bioactivities of their aqueous extracts, namely decoctions or infusions [11]. As regards chemical composition, most of the described studies have focused on organic extracts while less work has been performed to assess its phenolic composition.

However, to the best of our knowledge, no data are available in decoctions extracts or infusions extracts. Accordingly, the aim of the present study is to scrutinize the antioxidant, the *in vitro* and *in vivo* antifungal and the insecticidal activities of aqueous extracts of *Datura stramonium* and to compare them with extracts obtained by methanol extraction.

In this paper, and as a part of our investigations into some medicinal plants known in Tunisia we report the antioxidant, antifungal and insecticidal activities of aqueous and methanolic extracts of *Datura stramonium*. The antioxidant activity radical scavenging activity using 2,2-diphenyl-1-picryl hydrazyl (DPPH) assay to find possible sources for future novel antioxidants in food and pharmaceutical formulations. The in vitro and in vivo antifungal potential against *Fusarium oxysporum* and *Fusarium solani* was verified respectively by the filter paper disc technique and blotting paper method. While Repellent activity was evaluated by repellency test against adults of the red flour beetle *Tribolium castaneum.*

MATERIAL AND METHODS

Plant collection and authentication extraction

The fruit, leaf and aerial parts of *Datura stamonium* were collected at the full flowering stage in the month of February 2018 from Sahloul, Sousse at the coast of Tunisia (**Table 1**). The taxonomic identification of plant samples was authenticated by the Center for the Defense of Cultures (Kalaa sghira, Sousse, Tunisia). Leaves, fruits and roots are air dried at room temperature, then slightly blended into fine powders for extractions.

Preparation of the methanolic and aqueous extracts

Methanolic extraction was performed by mixing the plant material (10g) in 200 ml of dissolvent at 25°C for 16 h, then, the extracts were filtered through Whatman N°4 paper. The dissolvent was evaporated at 74°C under diminished weight using a rotating vacuum evaporator and afterward further lyophilized [13].

Infusions were assessed by adding 200 ml of boiling distilled water to the sample (10 g) and were left to stand for 5 min at room temperature. Samples were then filtered under reduced pressure and finally lyophilized. Decoctions were performed by adding 200 ml of distilled water to the sample (10 g), heated and boiled for 5 min. The mixtures were left to stand for 5 min and then filtered under reduced pressure. Both of infusions and decoctions were frozen and lyophilized [14]**.**

Table 1: Phytochemical screening of *Datura stramonium* extracts (Aqueous and methanolic fractions).

	M			I			D		
Biomelocules	L	Fr	R	L	Fr	R	L	Fr	R
Saponins	-	-	-	-	-	-	-	-	-
Alkaloids	-	-	-	-	-	+++	-	++	+++
Tanins	-	-	-	-	-	-	-	-	-
Anthocyanins	-	-	-	-	-	-	-	-	-
Coumarins	±	-	-	±	-	-	±	-	-
Terpenoids	-	-	-	-	-	-	-	-	-
Carbohydrates	±	-	-	-	-	-	-	-	-
Quinones	-	-	-	-	-	-	-	-	-
Fatty Acids	-	-	-	-	-	-	-	-	-
Amino Acids	-	-	-	-	-	-	-	-	-

M: Methanolic extracts, I: Infusions, D: Decoction, L: Leaves, Fr: Fruits, R: Roots. (**-**): negative test, (±): weak positive test, (++): strongly positive test. (+++): very strongly positive test

Phytochemical Screening

The following phytochemicals assays were referenced to protocols described by several groups and carried out in triplicate [15].

***Saponins*:** One milliliter of the extract was vigorously shaken with water from distillation. The positive indicator was a stable, lasting froth for 20 minutes.

***Alkaloids*:** The extract (1 mL) was treated with 3-5 drops of Wagner's reagent (1.27 g of iodine and 2 g of potassium iodide in 100 mL distilled water) and observed for coloration or the formation of reddish-brown precipitate.

***Anthocyanins*:** We added 2 mL of HCl (2 M, 1 mL) and ammonia (4 M, 1mL) to the extract (1 mL), respectively. The change of color from pink-red to blue-violet shows that anthocyanins are present.

Coumarins: NaOH (2 mL, 10%) was added to 1 mL of extract and the development of yellow shading demonstrates the existence of coumarins.

***Terpenoids*:** The extract (2 mL) was added to acetic anhydride (2 mL) and concentrated H_2SO_4 drops. The Development of blue, green rings shows the existence of terpenoids.

Carbohydrates (Molisch's Test): Few drops of Molisch's reagent were added to the extract (1 mL), followed by 1 mL of conc. H_2SO_4 drops. The mixture was allowed to stand for two-three minutes. The formation of a red or dull violet colour at the interphase of the two layers was a positive test.

***Quinones*:** The extract (1 mL) was added to concentrated HCl drops and observed for the formation of yellow precipitate or colouration.

***Tannins*:** The extract (1 mL) was added to 2 mL of water followed by drops of dilute ferric chloride solution (0.1%). Formation of green to blue-green (cathechic tannins) or a blue-black (gallic tannins) coloration indicates the presence of tannins.

***Amino acids and proteins*:** The extract (1 ml) was treated with drops of ninhydrin solution (1 %) and placed in a boiling water bath. The mixture was allowed to stand for two minutes. The formation of purple colour was a positive indicator.

***Fatty acids*:** 0.5 mL of extract was mixed with 5 mL of ether. The mixture was allowed to evaporate on filter paper. The appearance of transparence on dried filter paper was a positive indicator.

Quantitative phytochemical analysis

***Total phenolic content*:** In order to determine the amount

of total phenolics in the extracts, the Folin-Ciocalteu procedure was used with gallic acid as the standard. 0.25 ml of the sample was mixed with 1.25 ml of Folin–Ciocalteu's reagent (diluted ten-fold), and 1 ml of Na_2CO_3 (75 mg/ml).The absorbance of the mixture was measured at 765 nm against a blank without the sample after incubation at 40°C for 40 min. All determinations were assessed in triplicate and quantification was performed on the basis of the standard curve of gallic acid. Results were expressed as milligrams of gallic acid equivalents (GAE) per gram of extracts [16].

***Total flavonoids*:** Jia et al method was performed to determine the content of with rutin as a reference compound. The extracts (250µl), distilled water (1250 µl) and sodium iodide Na2NO2 (75 µl 5%) were shaken. Then 150 µl of $AlCl_3$ (10%) was added and allowed to stand for 6 min before adding 500 µl of NaOH (1M) and 250 µl of distilled water. The mixture was stand to ambient temperature for 15 min; absorbance was then measured at 510 nm. The total flavonoids content was expressed in milligrams of catechin equivalent (CE) per gram of samples. Analysis of each sample was calculated in triplicate [17].

***Flavonols content*:** We used the $AlCl_3$ method described by Miliauskas and al to determine the content of flavonols. Briefly, a mixture of 500 µl of the plant extract, 500 µl aluminum trichloride (2%) and 1500 µl of acetate of sodium (5%) was prepared, shaken and allowed room temperature in obscurity for 2h30 min. The absorption at 440 nm was then calculated. The absorption of rutin solutions was measured under the same conditions. Standard rutin solutions were prepared from 0.05 g rutin. All determinations were carried out in triplicate. The amount of flavonols in plant extracts was measured in milligrams of rutin equivalents (RUE) per 100 g of extracts [18].

***DPPH radical scavenging activity*:** The free radical scavenging effect of the plant sample was performed using a stable DPPH (1,1 diphenyl-2-picrylhydrazyl radical) on the basis of the method reported by Kartal with certain adjustments to the process [19]. DPPH has a 517 nm dissolving band that disappears after the anti-radical compound has been reduced. In short, 180 µl, prepared daily in the dark and kept in the dark, of various extract concentrations (0,009-0,312 µg/ml) was added to 1620 µl DPPH. The absorbances were measured at 517 nm and corresponded to the extract capacity of the yellow colored diphenyl-picrylhydrazine to reduce stable radical DPPH. The antiradical activity was shown as IC_{50} which was the extract dose needed to induce inhibition at 50 percent. A lower IC_{50} value is a higher plant extract antioxidant activity. The ability to radically scavenge the DDPH was measured using this equation: % inhibition=[(A0–A1)]/A0x100**.** Where A0 was controlled absorption and A1 was test compound absorption.

Antifungal activity

***Fungal culture*:** The culture of *Fusarium* (*oxysporum* and *solani*) isolates used in this study were obtained from tomato plants showing typical crown and root rot symptoms in Chott Mariem (Sousse). The isolation of fungal pathogen was realized by planting plant tissues (surface-disinfected with 1% sodium hypochlorite for 2 min) on PDA (Potato Dextrose Agar) and they were allowed to incubation at (25 ± 2)°C for 5 days. Isolates were identified as *Fusarium oxysporum* ou *Solani* morphologically based on characteristics of the macroconidia, microconidia, phialids, chlamydospores and colony growth traits. By the pathogenicity tests, the forma specialis of this pathogen was identified [20].

***In vitro* antifungal activity by filter paper disc method:** All the samples were evaluated for their *in vitro* antifungal potential against Fusarium oxysporum and Fusarium solani using a Filter paper disc method as it was described by Fandohan et al. [21]. Briefly, 10 µl of methanolic and aqueous extracts were added to Sterile Whatman paper disks (6 mm diameter) at appropriate concentrations, 40mg/ml and 100mg/ml respectively. Sterile Whatman paper disks (6 mm diameter) are soaked with methanolic, aqueous extracts, and distilled water (negative control). These discs are then placed in the petri dishes. The inoculate dishes were incubated 5 days incubation at (25 ± 2)°C. Disks containing PDA with no extracts were inoculated to serve as negative control. For positive control, disks were inoculated with Tachigaren, an antifungal compound. For each, the treatment was repeated three times. The fungitoxicity of the extract in terms of percentage inhibition of mycelial growth was calculated using the the Ebbot formula T=(Dk-D0)/Dk x 100 with Dk: Diameter of the fungal colony of witness (in mm); D0: Diameter of the fungal colony in presence of the extract (in mm); T: inhibition rate of the growth of the percentage mycelium [22].

***In vivo* antifungal activity:** The germination of the treated tomato and chilli pepper seeds was evaluated by the method described by Qi [23]. The seeds were treated with fungi strains (10^7spores/ml) and then dried under shade and were retreated by our plant extracts at the following concentrations: 40 mg/ml and 100 mg/ml for methanolic and aqueous extracts, respectively. The seeds were then taken to test their germination by using blotting paper method. Ten seeds from each treatment were placed on Petri dishes of 9.0 cm diameter containing water soaked blotting paper. Each treatment was replicated thrice. Positive control in which the plant extract was replaced by a synthetic fungicide (Tachigaren) was used at 1ml/ l). Sterile distilled water was used as negative control. The well germinated seeds in each petridishe were counted after 7 days of treatment and expressed in percentages. Germination rate(%)=(Number of germinated seeds/ Number of seeds tested)*100

Repellent activity: The repellency of *Datura* extracts was evaluated according to of McDonald [24]. Half filter disks were prepared (Whatman n°40, 9 cm diameter) and 50 mg each was diluted in 1 ml of methanol at 5% concentration. The other half (control) was mixed with 200 µl methanol or distilled water. The volume of 200 µl of each concentration was treated separately to a half of the filter paper as uniformly as possible. Both the processed and control halves were left to dry out in the air for 10 minutes, as they were exposed. Each of the processed halves was placed on a control half disk with sticky tape, in a Petri dish (9 cm in diameter), in a longitudinal, edge-to-edge direction.

In the middle of each filter-paper circle, 20 adult insects were released and four replications were made for each extract. After several intervals (15 minutes, 30 minutes, 1 hour and 2 hours) , insects were counted that were settled at every half the paper filter disk. The counts were averaged by the following formulation: **PR=[(NC–Nt)/(NC +Nt]*100;** with NC: number of insects in control test; Nt: number of insects in the treatment test. The mean repellence value of each extract has been calculated from 0 to V: class 0 (PR ≤ 0.1%); class I (PR=0.1-12%), group II (PR=20.1-40%), class III (40.1-60%), class

IV (60.2-80%), and class V (80.1-100%). A total number of repellency classes have been assigned for each extract.

Statistical analysis: The experiment results were statistically analyzed by SPSS Statistics for Windows version 21,0,t student test and Duncan's multiple range test were used.

RESULTS

Phytochemical screening

Phytochemical screening is a set of methods of analysis of the natural organic substances of the plant. It can detect the presence of all active compounds; the presence of these compounds is attested by the formation of a precipitate or a color change after the addition of specific reagents: Results illustrated in the **Table 2** highlight the presence or absence of phytochemicals. No plant organ alone possesses a wealth of all chemical groups. However some are richer than others. Results revealed that all samples showed a weak to a strong presence of phytochemicals, and that the methanol phytochemical profile has a similar trend to aqueous extracts. From **Table 1**, it was observed that alkaloids were intensively present in aqueous extract of roots, whereas coumarins and terpenoids were present in methanolic and aqueous extracts of leafs. However, the other compounds were absent in both methanolic and aqueous extracts except carbohydrates for organic extract of leafs. Once the presence of the basic phytochemicals was qualitatively confirmed, we moved to the quantitative assessment.

Quantitative phytochemical analysis

The results obtained during quantitative analysis by spectrophotometry are reported in **Table 2**. Our results revealed a difference in the contents of the phenolic compounds for each extract studied. According to the **Table 3**, we noted that the DS leaves are richer in phenolic compounds than the other parts of the plant regardless of the type of extraction. The highest content is attributed to the organic extract of leaves that is equal to 88 mg ER / g ext for flavonols. On the other hand, the roots are the least rich; they have the lowest contents or even absent. In particular, the content of flavonoids is equal to 0 mg ECat / g ext for aqueous extract (decoction). Except in the organic extract which has the highest value in flavonoids equal to 15.5 mg ECat/ g ext. concerning the fruits, they revealed contents of phenolic compounds which are not significantly different to those of the leaves.

DPPH radical scavenging activity

The antioxidant capacity of the various extracts was deduced by determining the IC50. The lower the IC50 value is the greater is the antioxidant activity of a compound. From **Table 3**, we noted that the percentage of free radical inhibition for the extracts was higher than that of the standard for all the concentrations used. By comparing the different plant extracts to our positive control (ascorbic acid: vitamin C), we found that the aqueous leaf infusion extract has the lowest IC50 equal to 0.5 mg / ml. On the other hand, the aqueous extracts (by decoction or infusion) of the roots have the strongest IC50, which reaches 39.17 mg / ml. As a result, D.S leaves have the most important antioxidant power compared to other plant organs regardless of the extraction method.

Antifungal activity

In vitro antifungal activity by filter paper disc method: The study of the effect of the different extracts on the fungus *Fusarium oxysporum* and *Fusarium* solani by the filter paper disks technique allowed us to notice that the organic and aqueous extracts of the various plant organs inhibit the fungal proliferation in a very efficient way compared to a control fungicide. Indeed, the results illustrated in **Table 4**, indicate that for *Fusarium oxysporum*, fruits decoction and leaves methanolic extracts have the most important antifungal activity with inhibition of mycelial growth. In fact 33.3% and 32.5% of inhibition was assessed by 40 mg / ml of the organic extract and 100 mg / ml of the aqueous extract respectively. On the other hand, the aqueous extract by root infusion has the lowest antifungal potential. For *Fusarium* solani, results presented by **Table 4** showed that all extracts had antifungal activity, with inhibition of mycelial growth percentage very close to the percentage of positive control inhibition. The aqueous extract by root decoction has the most important/highest percentage which is equal to 19% (**Table 4**). For in vivo study, only the

Table 2: Quantitative analysis of Total Phenolic, Flavonoids and Flavonols content of different extracts (methanolic and aqueous) of *Datura stramonium.*

	D.S								
	M			**I**			**D**		
Phenolic compounds	L	Fr	R	L	Fr	R	L	Fr	R
Total Phenolic (mg GAE /g ext)	53.37 ± 0.24 b	21.34 ± 0.62 c	7.79 ± 0.48 f	64.49 ± 0.81 a	16.59 ± 0.34 d	4.47 ± 0.76 g	54.75 ± 0.57 b	14.64 ± 0.67 e	5.80 ± 0.57 g
Flavonoids (mg CE /g ext)	9.38 ± 0.13 bc	10.44 ± 0.95 abc	15.50 ± 7.59 a	13.12 ± 1.74 ab	5.38 ± 2.53 cd	7.59 ± 0.95 c	15.50 ± 1.90 a	1.58 ± 0.00 de	0.00 ± 0.00 e
Flavonols (mg RUE /g ext)	88.38 ± 4.38 a	45.88 ± 1.00 b	5.13 ± 0.38 e	31.25 ± 0.63 c	9.75 ± 1.50 e	3.63 ± 1.13 f	20.19 ± 0.19 d	8.81 ± 0.32 e	2.63 ± 0.50 f

D.S: *Datura Stramonium*, M: Methanolic extracts, **I**: Infusions, **D**: Decoction, **L**: Leaves, **Fr**: Fruits, **R**: Roots. **GAE**: Gallic acid equivalents; **CE**: Catchin equivalents; **RUE**: Rutin equivalents, **Ext**: extract. Different superscript letters in the same row indicate significant difference between values at $p < 0.05$ level (Duncan's multiple range test) and values are mean ± standard deviation

Table 3: Antioxidant assays (IC_{50} values) of different extracts (metabolic and aqueous).

		D.S								
	Vit C	M			I			D		
		L	Fr	R	L	Fr	R	L	Fr	R
IC50 (mg/ml)	0.005	0.50 ± 0.01 i	1.19 ± 0.01 f	6.16 ± 0.01 c	0.70 ± 0.01 h	3.28 ± 0.01 e	39.17 ± 0.01 a	1.03 ± 0.06 g	3.60 ± 0.01 d	19.62 ± 0.01 b

D.S: *Datura Stramonium*, M: Methanolic extracts, **I**: Infusions, **D**: Decoction, **L**: Leaves, **Fr**: Fruits, **R**: Roots. **GAE**: Gallic acid equivalents; **CE**: Catchin equivalents; **RUE**: Rutin equivalents, **Ext**: extract. Different superscript letters in the same row indicate significant difference between values at $p < 0.05$ level (Duncan's multiple range test) and values are mean ± standard deviation

aqueous extract (decoction) of roots and methanolic extracts of leaves and roots have been used since they have the best *in vitro* antifungal potential.

In vivo antifungal activity: The results presented in **table 5** showed that the aqueous and organic root extracts gave the percentage of germination of tomato seeds infected with Fusarium oxysporum at a concentration of 100 mg/ml and 40 mg/ml respectively. However, the aqueous and organic root extracts do not stimulate the germination of chilli pepper seeds; they have the lowest percentage. Methanolic preparation of leaves presented activity only on tomato seeds infected with only Fusarium oxysporum although Fusarium oxysporum and Fusarium solani belong to the same genus.

Repellent activity

In this study we evaluated the repellent effect of organic and aqueous extracts of *Datura stramonium* on *T. castaneum*, insect of stored food. The results obtained in **Table 6** showed that the aqueous extracts by infusion of the three tested organs of DS all have a repellent activity on the adults of *Tribolium castaneum.* The most remarkable effect is recorded by the root after only 15 min of exposure (%) which belongs to the repulsive class II, followed by the leaf extract (27.5%, class II), and at the end the fruit extract which gives the lowest percentage of repulsion after the same exposure time (12.5%, class I). For the three organs, it is found that after 2 hours the repulsion percentage decreases. For aqueous extracts by decoction, we noted that the leaves have

Table 4: Effect of the different extracts on *Fusarium oxysporum* and *Fusarium solani.*

Fusarium oxysporum											
			M			**I**			**D**		
	T(+)	T(-)	L	Fr	R	L	Fr	R	L	Fr	R
% Inhibition	4.7	0	33.33 ± 2.38 a	N.D	26.98 ± 2.75 ab	24.60 ± 9.91 ab	29.37 ± 5.50 ab	12.70 ± 6.87 c	21.43 ± 6.30 bc	32.54 ± 5.50 ab	26.19 ± 4.12 ab
Fusarium solani											
			M			**I**			**D**		
	T(+)	T(-)	F	Fr	R	F	Fr	R	F	Fr	R
% Inhibition	14.2	0	14.29 ± 0.00	ND	17.14 ± 4.95	15.24 ± 1.65	16.19 ± 3.30	14.29 ± 4.95	15.24 ± 1.65	13.33 ± 1.65	19.05 ± 8.25

D.S: *Datura Stramonium*, M: Methanolic extracts, **I**: Infusions, **D**: Decoction, **L**: Leaves, **Fr**: Fruits, **R**: Roots. **T(+)**:positive test, **T(-)** :negative test, **ND**: not determined. Different superscript letters in the same row indicate significant difference between values at $p < 0.05$ level (Duncan's multiple range test) and values are mean ± standard deviation

Table 5: *In vivo* antifungal activity.

Fusarium solani				
	Tomato seeds	**Chilli pepper seeds**	**Tomato seeds**	**Chilli pepper seeds**
Aqueous (Decoction) root extract	73.33 ± 5.77	20.00 ± 0.00	-	-
Organic root extract	66.67 ± 5.77	6.67 ± 11.55	-	-
Organic leaf extract	-	-	50 ± 20 *	0

Values are mean ± standard deviation. * indicate significant difference between values at $p<0.05$ level (t student test)

Table 6: The percentage of repulsion of *T. castaneum* adults exposed to aqueous extracts of DS.

Infusion				
Exposure time	**Organ**	**Mean repellency (%)**	**Repellency Class**	**Effect**
15 min	L	27.5 ± 0.1 b	II	Low repulsion
15 min	Fr	12.5 ± 0.1 c	I	Very Low repulsion
15 min	R	32.5 ± 0.2 a	II	Low repulsion
30 min	L	27.5 ± 0.3 a	II	Low repulsion
30 min	Fr	20.0 ± 1.0 c	I	Very Low repulsion
30 min	R	17.5 ± 0.1 d	I	Very Low repulsion
1 h	L	22.5 ± 0.2 b	II	Low repulsion
1 h	Fr	25.0 ± 2.0 b	II	Low repulsion
1 h	R	32.5 ± 0.4 a	II	Low repulsion
2 h	L	20.0 ± 1.5 b	I	Very Low repulsion
2 h	Fr	7.5 ± 0.5 c	I	Very Low repulsion
2 h	R	17.5 ± 1.5 b	I	Very Low repulsion
Decoction				
Exposure time	**Organ**	**Mean repellency (%)**	**Repellency Class**	**Effect**
15 min	L	-	-	-
15 min	Fr	-	-	-
15 min	R	-	-	-
30 min	L	22.5 ± 0.2 b	II	Low repulsion
30 min	Fr	-	-	-
30 min	R	-	-	-
1 h	L	17.5 ± 1.5 c	I	Very Low repulsion
1 h	Fr	-	-	-
1 h	R	-	-	-
2 h	L	57.5 ± 0.2 a	III	Moderate repulsion
2 h	Fr	-	-	-
2 h	R	-	-	-

L: leaves, Fr: fruits, R: roots, H: hours, Min: minutes. Different superscript letters in the same row indicate significant difference between values at $p<0.05$ level (Duncan's multiple range test) and values are mean ± standard deviation

a repellent effect against *T. castaneun*. This effect was recorded only after 30 min of exposure to reach the highest repulsion percentage of 57.5% after 2h. With regards to methanolic extracts No effect was observed with organic extracts.

DISCUSSION

Plants represent unlimited source of primary and secondary metabolites. Secondary plant metabolites are of major interest because of their different functions and the impressive range of their biological activities [25]. Herbs belonging to the Solanaceae family are one of these sources. This diversity in biological properties is certainly related to the therapeutic virtues attributed to an extraordinary range of bioactive molecules synthesized by the plant not only as chemical agents against diseases, herbivores and predators but also as medicinal agents such as antioxidants. These natural molecules of phenolic nature are very popular in herbal medicine for the side effects of drugs and pesticides, because their importance in the medicinal and agricultural sectors is manifested by their antioxidant, antifungal, insecticidal properties. This work represents a comparative study of two different aqueous extracts (decoction and infusion) and methanolic extracts of *D. stramonium* for their biological activities in order to valorize this toxic plant. The analysis of the chemical composition of these two extracts was carried out as well as the determination of their chemical composition. We also studied their antioxidant, antifungal (*in vitro* and *in vivo*) and insecticidal properties.

Phytochemical screening of leaf extracts of *D. stramonium* showed positive results only for coumarin coumpounds, whereas the research of Ananth and his collaborators revealed the presence of tannins, saponins, flavonoids and alkaloids in aqueous extract leaves. In fact, the presence of phenolic compounds enables plants to act as reducing agents which could explain the antioxidant, the antifungal and the insecticide activities of this studied species [26]. Polyphenols was found in all the samples and in the following order: Leaves > fruits> root. According to the study of Mahinder and his collaborators, we have noticed that our methanolic extract of roots is the richest on total phenolics, (6.5 mg GAE/ g dry ext and 7. 7 mg GAE/g Ext) and on flavonoids contents (2.28 mg CAT /g dry ext and 15.5 mg CAT/g ext) [27].

Antioxidants are enormously important substances which have the capability to defend the body from damage engendered by free radical induced oxidative stress. The antioxidant potential of *D. stramonium* methanolic extracts was investigated in the search for new bioactive compounds from natural resources. The study elaborated by Ananth and his collaborators showed the highest antioxidant activity by DPPH assay of aqueous extract of *D. stramonium's* leaves (0.08mg/ml; 0.5 mg/ml) [26]. Phenolics are the mostly wide spread secondary metabolite in the plant kingdom. These diverse groups of compounds have potential of natural antioxidant and have ability to act as both efficient radical scavengers. The antioxidant activity of phenols is due to their redox properties, hydrogen donors and singlet oxygen quenchers**.** The compounds such as flavonoids; which contain hydroxyls are responsible for the radical scavenging activity in plant [26]**.** On the basis of these results it is concluded that the extracts containing higher quantities of phenolic compounds, exhibit antioxidant and free radical scavenging activities.

Regarding the antifungal activity *in vitro*, the obtained extract inhibited radial mycelial growth of all the test fungi at a concentration of 40 mg/ml for aqueous extract and 100 mg/ml for organic extract to varying degrees that ranged from 13.3% to 33.3%. According to these results, both of methanolic and aqueous extracts of *D. stramonium* had the best antifungal activity against all the tested strains of *Fusarium*. However, no effect on F*usarium solani* was found for in vivo antifungal although they belong to the same genus. The antifungal activity of plant extracts may be associated with the presence of certain bioactive compounds such as phenolic compounds, flavonoids, polyphenols and tannins [28]. Some studies have shown that the antifungal effect could be explained by the enzymatic inhibition exerted by the phenols derived from the oxidation compounds and by the stimulation of synthesis of the inhibition proteins in the cell by tannins [29]. The results reported in this experiment are completely in accordance with several studies already published showing the potent antifungal activity *Datura stramonium* compared to those tested in the present study. For example, Arzoo ve Biswas claimed that *Datura stramonium* L. aqueous extract inhibited mycelial growth of *Fusarium oxysporum f.sp. lycopersici* by 13% [30]. Mdee LK and al have reported that acetone extracts of *D. stramonium* have antifungal activity against *Fusarium oxysporum* [6]. Shinde and Dhale investigated the antifungal activity of *Datura stramonium* L. extracts on *Fusarium oxysporum*. According to this study, *Datura stramonium* L. stem bark alcoholic extract showed maximum antifungal activity. In the plants parts, the most antifungal effect on fungi was at stem bark extract and then was at leaves and root bark respectively [31]. Biopesticides (leaf extracts) obtained from *D. stromonium* showed antifungal activities against the fungal pathogen (*Fusarium oxysporum*) of wilt of pigeon pea (*Cajanus cajan* L.). Both *in vivo* and *in vitro* higher concentration of ethanotic leaf extracts of all eight plants showed complete inhibition in linear growth and sporulation in test fungi [12]. However, very little research has been done specifically on the antifungal activity of *Datura stramonium* extracts against fungi, especially for aqueous extracts. To the best of our knowledge, no previously reported data have shown the *in vivo* anifungal activity of aqueous and methanolic extracts on the on the germination of tomato and chilli seeds.

Concerning insecticidal activity, the results of this research showed that maximum repellency against *Tribolium castanum*, was found in aqueous extract of leaves after 2 hours. The toxic and repellent effect of this plant may depend on its chemical composition and the level of sensitivity of insects. All parts of the plant (Datura) contain alkaloids: hyoscyamine, atropine and scopolamine. Their quantities and proportions vary according to the species considered, the part of the plant as well as the environmental conditions. Datura plants have a total alkaloid content of 0.2% to 0.6%; the third is scopolamine; the remaining 2 thirds of hyoscyamine and atropine. Young plants would be richer in scopolamine than adult plants [32,33]. However, the extract of *D. stramonium* is composed of certain alkaloids (such as scopolamine, hyoscyamine, meteloidine, and apoatropine), terpenoids, and flavonoids that are believed to be responsible for many of this plant's insecticidal properties [34]. The study of Tirfanu, et al. showed that ethanolic *D. Stramonium* extract had a 96.30% repellent effect on *T. castaneum* adults after 12 h [35]. On the other hand, the study elaborated by S. Modarres Najafabadi shows that the ethanolic extracts of plant leaves

Datura showed maximum repellency of 73.33 ± 4.12% against *Tribolium castanum* after 24 h [36].

CONCLUSION

In the light of all the interesting results obtained, it is possible to hope for at least a reduction in the use of chemical pesticides .This toxic plant of Tunisia can be used as an alternative method to control several pests such as insects and pathogens through their biopesticides activities. Thus, in this study we have clearly shown that the use of aqueous extract could be a better and more promising source of antifungal, insecticidal and antioxidant agents than methanolic extracts. Devoid of toxicity and being rich in phenolic compounds, either phenolic acids or flavonoids, aqueous extracts could provide considerable effect against fungi and pests. They also could be used as a natural method for the extraction of antioxidants, especially since methanol is a toxic solvent. Nevertheless, further experiments are required in order to purify, explore the mechanisms of action involved and elucidate the structure of active compounds for the development of a new class of biological metabolites.

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