Physicochemical study, phytochemical screening and dosage of phenolic compounds of the *Solanum elaeagnifolium* plant

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Abstract- *Solanum elaeagnifolium* (yellow nightshade) is a herbaceous plant that belongs to the *Solanaceae* family, which are herbaceous plants, trees, shrubs or lianas widely distributed in tropical regions. The objective of this work is to highlight the existence of secondary metabolites that characterize *Solanum elaeagnifolium*. To achieve these objectives, a qualitative study based on phytochemical screening was carried out on the different parts (leaves, fruits, stems and roots) to highlight the existence of secondary metabolites. The results of this study showed that the leaves are rich in flavonoids, tannins, coumarins, reducing sugars and irridoids, the fruits are rich in Flavonoids, Tannins, coumarins and reducing Sugars, the Stems are rich in coumarins, reducing sugars and the roots are rich in flavonoids, reducing and irridoid sugars. The dosage allowed us to deduce that the phenolic composition varies according to the part studied. Indeed the fruits showed the highest contents of total polyphenols (5.129 ± 0.40 mg/g dry matter) Concerning the total flavonoids, the highest contents were determined in the leaves (0.618 ± 0.01 mg/g dry matter) followed by the fruits (0.460±0.01 mg/g dry matter). Similarly, the fruits (1.380±0.97 mg/g dry matter) were the richest in tannins followed by the stems (0.506±0.73 mg/g dry matter); leaves (0.431±1.57 mg/g dry matter); and roots (0.251±1.37 mg/g dry matter).

Keywords: *Solanum elaeagnifolium, physicochemical; screening; dosage.*

I. INTRODUCTION

The yellow nightshade (*Solanum elaeagnifolium* Cav. simply called # SOLEL), also called silverleaf nightshade, is a Solanaceae considered in its area of origin (southern United States and northern Mexico), as a weed [1] and outside this area as an Invasive Alien Plant [2]. Is a perennial that has become more economically problematic in recent decades. The intensive use of soil-applied herbicides, along with reduced competition from annual weeds and reduced soil maintenance, have contributed to its spread and establishment as a serious plague. Crop plants are either directly affected via competition and allelopathy, or indirectly by destructive herbivorous insects or pathogenic fungi hosted by Solanaceae
It is toxic to certain livestock [3]. In addition, its invasion seriously unbalances natural ecosystems [4].

II. Material and Methods

II.1 Vegetal material

The plant used in this study was randomly harvested from Rabat region in January 2022. The whole plant is pulled by hand. The leaves, fruits, stems and roots of *Solanum elaeagnifolium* are dried in the shade, away from humidity and the sun, in order to retain as many molecules as possible, then ground using a electric grinder and carefully preserved the different parts of the plant.

II.2 Physicochemical analyses

1. Moisture determination

The method used for the determination of moisture is based on that proposed by AOAC [5], the principle of which is based on the loss of mass of the sample to a constant mass at 105°C.

2. pH determination

The measurement of pH was determined using the method [6].

3. Determination of total ash content

It is a question of evaluating the quantity of residual substances not volatilized when the drug is completely calcined. From the drug powder used to measure the water, introduce a test portion of 2 to 3 g into three

previously weighed crucibles. Calcine in the oven at 600°C for 6 h, allow to cool to laboratory temperature [7].

Test drug mass = mass before calcination - tare

Ash mass = mass after calcination - tare

$$\% \text{ ash total ash} = \frac{\text{Ash mass}}{\text{Drug test mass}} \ X\ 100$$

II.3 Phytochemical screenings

The preliminary phytochemical screening aims to characterize the different chemical groups present in each part. It was carried out on extracts by characterization reactions in tubes. It is a qualitative method which is based either on the formation of insoluble complexes by precipitation reactions, or on the formation of colored complexes by coloring reactions (conjugation or unsaturation in a molecule) [8].

1. Characterization of Phenolic Compounds

Preparation of filtrate: an infusion is prepared from 5 g of powder sample added to 100 ml of boiling water and allowed to stand for 15 min, it is then filtered and adjust with hot water up to 100 ml. The extract obtained will be used for the characterization of tannins and flavonoids.

a- Characterization of tannins

Free tannins at 5ml are infused at 5% and 1 ml of FeCl 3 aqueous solution at 1 % are introduced into a tube. The development of a blackish or greenish blue color indicates the presence of tannins [9].

Differentiation

* The reaction Stiasny : it consists of adding 30 ml of 5% infused with 15 ml of Stiasny reagent and then heated in a water bath at 90 ° C for 15 min. Obtaining a precipitate shows the presence of catechol tannins.

Filter the filtrate saturated with sodium acetate, after adding 1 ml of 1% FeCl3. The development of a blue-black color signals the presence of gallic tannins not precipitated by Stiasny reagent [9].

1.2. Characterization of flavonoids

a. Response to cyaniding

1 ml infused at 5%, 1 ml hydrochloric alcohol and 1 ml of isoamyl alcohol are introduced into a tube by adding some magnesium turnings. Crepitus reaction occurred for a few minutes [10].

The appearance of the isoamyl alcohol supernatant layer of a colouring:

- Orange Rose characterizes flavones.

- Purple Rose characterizes flavanones.

- Red characterizes flavanones and flavanonols.

b. Anthocyanins

This test is performed by adding 5 ml of infused to 5%, 5 ml of sulfuric acid and 5 ml of NH 4 OH in a tube. In the presence of anthocyanins, the colour deepens by acidification and then turns purplish blue in basic medium [10].

c. Leucoanthocyanins

Cyanidin reaction is heated in a water bath for 15 minutes without the addition of magnesium shavings. In the presence of leucoanthocyane, it develops a cherry red colour with purplish or against the red-brown colour indicates the presence of catechol [10].

1.3. Characterization of Quinones

An amount of 0.5 g of the powder sample was introduced into 5 ml of petroleum ether and stirred for a few minutes; the resulting mixture was rested for 12 hours. After filtration, the extract is evaporated to the rota-vapor. The colour change of the aqueous phase in yellow, red or violet after adding a few drops of NaOH (1/10) is proving the presence of quinones [11].

2. Characterization of sterols and terpenes

Preparation of the filtrate: 1 g of powder sample is digested in 20 ml ether, then stirred and left in the dark for 24 hours. After filtration, the filtrate was adjusted to 20 ml with ether. Sterols and terpenes are highlighted by adding 10 ml of the etheric extracted with 1ml of $CHCl_3$ and 1 to 2 ml of concentrated H_2SO_4 in a tube. The cookie contains only $CHCl_3$ and the extract. The formation of a brownish red or purple

ring at the two liquid contact zones, reveal their presence [12].

3. Characterization of alkaloids

An amount of 2 g of sample powder is saturated in 15 ml of distilled water for 24 hours in the dark and then filtered. A few drops of Dragendorff reagent are added to 1 ml of the aqueous extract. The presence of alkaloids is marked by the formation of an orange precipitate.

4. Characterization of saponosides

2 ml of aqueous extract diluted to half in distilled water in a tube are stirred continuously for 15 seconds. The persistence of foam of at least 1 cm for 15 minutes indicates the presence of saponins [13].

5. Protein characterization

Biuret reaction: maceration is prepared by a 1g of powder samples in 10 ml of methanol for 24 h in the dark and then filtered. 2 -3 drops of an aqueous solution of CuSO4 to 2% are added to an aliquot of residue dissolved in 2 ml of 20 % aqueous NaOH. The appearance of a purple colour, sometimes with a reddish tinge, indicating the presence of proteins [14]

6. Characterization derivative anthracene:

6.1 Characterization of free anthraquinone

10 ml of chloroform and samples of 1g powder are heated for 3 minutes. After hot filtration, 1 ml of the extract was added to 1 ml of diluted NH4OH. After shaking, the more or less red colour indicates the presence of free anthraquinones [15].

6.2 Anthracenes characterization

Combined O -glycosides: A hydrolyzate is prepared from the residue samples exhausted with chloroform which are added to 10 ml of water and 1 ml of concentrated hydrochloric acid. After boiling for 15 minutes in a water bath, 5 ml of the hydrolyzate is added to 5 ml of chloroform. After decanting, 1 ml of diluted NH 4 OH is added to the organic phase. A more or less intense red color indicates the presence of genins O -glycosides [15].

C -glycosides: The sample solution is the aqueous phase obtained from the O- glycosides solution. 10 ml of water and 1 ml of $FeCl_3$ is added to this solution, then heated in a water bath for 30 minutes. After cooling, 5 ml of $CHCl_3$ are added to the solution. Drawing off the chloroform layer and adding 1 ml of diluted NH_4OH, the appearance of a more or less intense red color indicates the presence of genins C-glycosides [15].

7. Characterization of mucilage

1 ml of 10% aqueous decoction is added to 5 ml of absolute ethanol. Mucilage are characterized by the appearance of a flocculent precipitate [12].

8. Characterization of reducing sugar

5 ml aqueous decoction are introduced into a tube and evaporated in a water bath until dry. To the residue is added 1 ml of Fehling reagent. Obtaining a brick red precipitate indicates the presence of reducing compounds [16].

9. Characterization iridoides

1 ml of a decoction of the aqueous extract to 5% is added to 1 ml of concentrated hydrochloric acid. The formation of a black precipitate after heating characterizes the presence of iridoids [17].

II.4 Determination of phenolic compounds by the colorimetric method

1. Assay of total polyphenols (PPT)

The total phenolic compounds of the extracts were determined using the Folin-Ciocalteu procedure [18] and [19], 20 µL of sample added to 1.58 ml of distilled water, 100 µl of Folin-Ciocalteu's reagent Ciocalteu and mixed with 300 µL of sodium carbonate (7.5%). The mixture was heated for 30 min at 45°C. Absorbance was measured at 764 nm after cooling to room temperature. The total content of phenolic compounds was expressed in mg of gallic acid equivalents per g of samples (mg GAE/g).

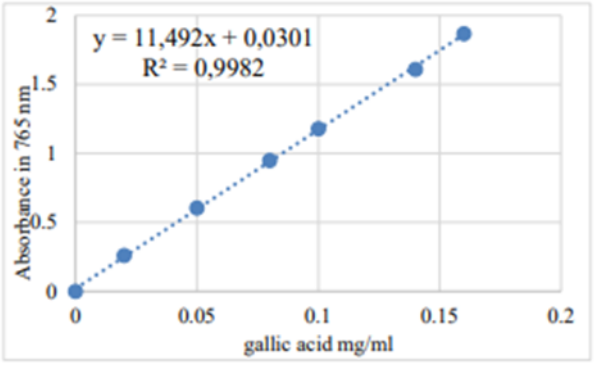

Figure 1: Calibration curve of gallic acid for the determination of total polyphenols

2. Assay of total flavonoids

The content of total flavonoids was carried out according to the method reported by [20]. This method consists of mixing 1 ml of the sample with 1 ml of $AlCl_3$ solution (2%). Samples were incubated for 1 h at room temperature. Absorbance was determined using a spectrophotometer at 415 nm. Total flavonoids were expressed as quercetin equivalents by reference to the standard calibration curve (mg QE/g).

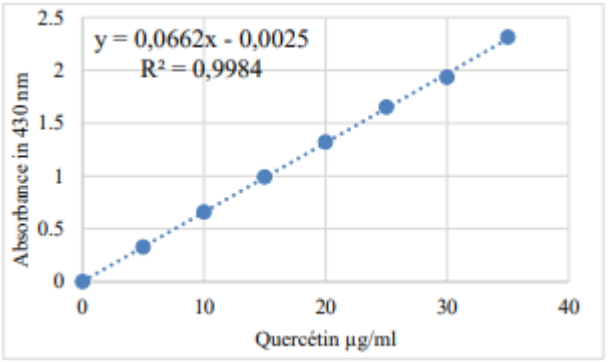

Figure 2: Quercetin Calibration Curve for Total Flavonoids

3. Dosage of condensed tannins

Condensed tannins were determined by the vanillin method in an acid medium as the method by [21]. Thus, an aliquot of 200 µL of each sample was added to the vanillin reagent (8% HCl, 37% methanol and 4% methanolic vanillin). Then the tubes were placed in a water bath at 30°C for 20 min. This method is based on the ability of vanillin to react with condensed tannins in the presence of acid to produce a colored complex measured at 500 nm. The results were expressed in mg of catechin equivalents per 1 g of sample (mg CE/g).

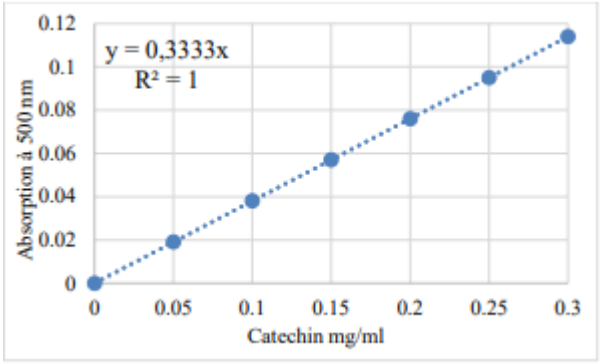

Figure3: Catechin calibration curve for condensed tannin determination

4. Statistical analysis

The results of the tests carried out are expressed as mean ± SD. Graphical representations of the data were made using Excel 2013 (Microsoft U.S.A).

III. Results and discussion

III.1. Physicochemical analyzes

The pH of the different parts of *Solanum eleangfollium* made it possible to obtain the values recorded in Table 1. These values show that the higher value in the leaves and for the roots and stems have a value almost equal to that of the leaves. However, the lowest value is indicated in fruits.

Table 1: Results of physicochemical tests of different parts of *Solanum elaeagnfolium*

	Leaves	Fruits	Stems	Roots
pH	5.997	4.953	5.180	5.522
Water content (%)	0.319	0.216	0.177	0.222
Total ash (%)	11.19	4.36	6.61	29.51

The pH in the different parts of *Solanum eleangfollium* made it possible to obtain the values recorded in Table 1. These values show that the higher values in the leaves and for the roots and stems have a value almost equal to that of the leaves. However, the lowest value is indicated in fruits.

The dosage of ash in *Solanum eleangfollium* made it possible to obtain the values recorded in table 1. These values show that the ash content of different parts of the plants is relatively higher in the roots; however the lowest values are indicated in fruits and stems.

III.2 Phytochemical screening

The results of the phytochemical screening showed the presence of polyphenols in the leaves and fruits, coumarins in the leaves, fruits and stems, C-glycosides in the fruits, mucilage in the leaves, fruits, stems and roots, reducing sugars in the leaves fruits stems and roots and for the irridoids are concentrated at the level of the leaves and roots. The phytochemical investigation of the extracts of *S. elaeagnifolium* is presented in the table below.

Table 2: Preliminary phytochemical screening of different parts of *Solanum elaeagnifolium*

		Leaves	Fruits	Stems	Roots
Polyphenols		+++	++	-	-
Free tannins		+++	++	-	-
Catechic tannins		-	-	-	-
Gallic tannins		+++	++	-	-
Flavonoids	Cyanidine	+++	++	-	+
	Anthocyanins	+++	++	-	+
	Leucoanthocyanins	-	-	-	-
Quinones		-	-	-	-
Sterols and terpenes		-	-	-	-
Coumarin		+	+	+	-
Alkaloids		-	-	-	-
Saponosides		-	-	-	-
Protein		-	-	-	-
Free anthraquinone		-	-	-	-
O –glycosides		-	-	-	-
C –glycosides		-	+	-	-
Mucilage		+	+	±	+
Reducing sugar		+	+	+	+
Irridoides		+	-	-	+

+++: Strong positive reaction; ++: Positive reaction; +: weak positive reaction; ±: some traces; -: Negative reaction

While the phytochemical study that was made on S. nigrum [22] showed that the aqueous extract is low in saponin while the hexane and benzene extracts are rich in saponins, tannins are present in the extracts. Benzene chloroform ethanol and aqueous but absent in the hexane extract. And for the alkaloids are present at the level of the chloroform extracts of ethanol and aqueous and yet absent at the level of the extracts of hexane and benzene. For phytosterols are present at the level of hexane, benzene, chloroform, ethanol and aqueous extract. The flavonoids are present just at the level of ethanolic and aqueous extract. For coumarins are present at the level of ethanol and aqueous chloroform extract.

III.3 Determination of phenolic compounds by the colorimetric method

The results, presented in figure 4, show that the content of total polyphenols varies according to the part analyzed. Indeed the fruits showed the highest PPT contents (5.129 ± 0.40 mg/g DM). These results remain lower than those found by Ouerghemmi et al. (2017), (367.60 mg EAG/g DM and 384.51 mg EAG/g DM for stage 1 and stage 2 respectively). Indeed, the roots showed the lowest levels of total polyphenols.

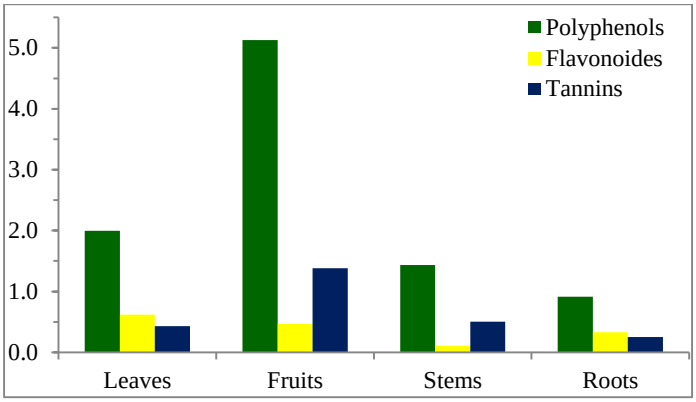

Figure 4: Results of assays of different parts of *Solanum elaeagnifolium*

Regarding total flavonoids (TF), the highest levels were determined in the leaves (0.618 ± 0.01 mg/g DM) followed by the roots (0.330 ± 0.02 mg/g DM). These results are lower than those found by OUERGHEMMI et al (2017) [23], who found 304.71 mg EC/g DM and 298.54 mg EC/g DM for stages 1 and 2 respectively.

Similarly, the fruits were the richest in tannins (1.380±0.97 mg/g DM) followed by the stems (0.506±0.73 mg/g DM), the leaves (0.431±1.57 mg/g DM) and the roots (0.251±1.37 mg/g DM). These results are lower than those found by Ouerghemmi et al. (2017), who found 434 - 342.9 mg EC/g DM of mature and immature fruits respectively. Thus, the results enabled us to clearly deduce the variation in the contents of PPT, FT and condensed tannins (TC) according to the organ tested, leaves, fruits, stems and roots.

These differences due to the change of geographical position, climate, the solvent and the technique used, the sample tested and depending on the stage of maturation.

IV. Conclusion

A phytochemical study followed by the quantification of the total polyphenols of the aqueous extract of the different parts of *Solanum elaeagnifolium* for a characterization of the chemical substances likely to be exploited in several fields such as pharmaceutical, food, cosmetics... The extraction of these compounds is an essential step for the valorization of these active ingredients, it depends on the method and the appropriate solvent and the part studied. The study proved that the infusion makes it possible to identify and quantify secondary metabolites. The results enabled us to clearly deduce the variation in the

contents of total polyphenols, flavonoids and condensed tannins according to the organ (leaves, fruits, roots and stems).

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