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Evaluation of Antimicrobial and Antioxidant Activities of Methanolic Extracts of Flavonoids Obtained from the Leaves of Solenostemma argel Plant Collected in the Region of Tamanrasset, Algeria

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Abstract

The objective of this study is to evaluate the antioxidant activity and antibacterial activity of the methanolic extracts obtained by using organic solvents with increasing polarity of leaves of *Solenostemma argel*, a medicinal plant of the traditional pharmacopeia of Tamanghasset (great Sahara) of Algeria. Initially, after extraction obtained by maceration of *S. argel*, the amount of total flavonoids isolated in the four extracts (ether, ethyl acetate, butanolic and aqueous extracts) was given, and by thin layer chromatography, profiles of each extract is obtained. The proportion of the flavonoids of these same extracts showed the wealth of this plant on total flavonoids. The antioxidant activity was evaluated by using 2, 2-Diphenyl-1-picrylhydrazyl (DPPHï) radical scavenging assay and the antibacterial effect of these extracts was evaluated by using the agar diffusion method on three bacterial strains. The results reveal that, only the ether extract of the plant collected in the area of Adrian (AD) exerted a considerable antibacterial effect only against Pseudomonas aeruginosa with zones of inhibition (09 mm and 08 mm). With final, the extracts of *S. argel* have an excellent antioxidant activity and a weak antibacterial effect.

Keywords: *Solenostemma argel*; Flavonoids; Antioxidant activity; DPPH; Antibacterial effect

Introduction

Herbal medicine sometimes referred to as herbalism or botanical medicine, is the use of herbs for their therapeutic or medicinal value. An herb is a plant or a plant part valued for its medicinal, aromatic or savory qualities. Herb plants produce and contain a variety of chemical substances that act upon the body [1]. It has been estimated that approximately 80% of the world's inhabitants rely mainly on traditional medicines for their primary health care; where plant based systems still play a vital role in health care. In developed countries, plant drugs are also extremely important, currently at least 119 chemicals derived from plant species can be considered as important drugs in use [2]. Spices and herbs have been used for thousands of centuries by many cultures to enhance the flavor and aroma of food. Early cultures also recognized the value of using spices and herbs in preserving food and for their medicinal value [1]. Scientific experiments since the late 19th century have documented the antimicrobial properties of some spices, herbs, and their components [3].

The plant *Solenostemma argel* is a member of the family Asclepiadaceae that comprises numerous medicinal plants, like *Calotropis procera, Marsdenia obyssinicna* and *Huernia mecrocarpa*, known for their cardiac activity. Tamanrasset is situated in the south of Algeria, in the mountains of the Hoggar to 1400 m altitude. This city is in the heart of the Sahara, the biggest hot desert in the world.

S. argel is a Saharan species; it grows naturally in the Tassili n'Ajjer, in Tassili Hoggar, in the Tadrart. It is also widely distributed throughout North Africa (Egypt, Libya, Algeria and Sudan) and the Saudi Arabia [4]. Leaves of this plant are used in indigenous medicine for the treatment of some diseases such as the disease of liver and kidney. It is an effective remedy for bronchitis and is used to treat neuralgia. It is used as incense in the treatment of measles and sometimes crushed and used as remedy for healing wounds. The leaves are infused to treat gastro-intestinal cramps and stomach colic.

The present study aimed to investigate the antioxidant and antibacterial activities of the methanolic extracts, it is to develop:

The extraction of total flavonoids from the leaves of *Solenostemma argel*;

The quantification of flavonoids by a spectrophotometric method;

Qualitative analysis of different extracts obtained by thin layer chromatography (TLC);

And finally, the evaluation of antioxidant activity (the test using the radical DPPH) and antimicrobial effects (the antibacterial test against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* which can be pathogenic to humans).

Materials and Methods

Collection of samples

Samples of *Solenostemma argel* leaves were harvested in the region of Adrian that located in Tamanrasset, during 2015. The leaves were freed from foreign materials like stones, sand and dust, before kept in the Lab., for further investigation. The leaves were then washed with water, dried, and milled using laboratory mill into fine powder.

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Source of cultures

The bacterial strains used in the antibacterial assay are: Gram-bacteria (*Escherichia coli, Pseudomonas aeruginosa*) and Gram+bacterium (*Staphylococcus aureus*). They come from the microbiology laboratory of hospital of Tamanrasset, and were stored at 4°C in drinking dish containing the solid medium Mueller Hinton until use.

Methods

Preparation of flavonoids extracts: The extraction of total flavonoids (TF) from *Solenostemma argel* is a liquid-liquid extraction based on the principle of solubility in organic solvents. The choice of solvent depends on the nature of the compounds to be extracted, their solubility in the solvent and especially the nature of the plant material.

It is based on the degree of solubility of the polyphenols in organic solvents. Therefore, three organic solvents of different polarities were used (diethyl ether, ethyl acetate and n-butanol) according to the extraction protocol described by Merghem et al. [5] with some modifications. 5 g of dry plant made powder placed in a container containing a solvent mixture (methanol: water) (70:30), the sample is allowed to macerate for 48 hours at room temperature. This operation is repeated three times. After filtration on filter paper, the filtrate was evaporated almost to dryness using a Soxhlet (45-50°C). The dry residue is taken up in boiling distilled water.

After stirring, the aqueous phase will undergo clashes with solvents of increasing polarity: (Ether, ethyl acetate and n-butanol).

The various recovered phases (ether, ethyl acetate, butanol and the residual aqueous phase) are evaporated to dryness then taken up with 5 ml methanol. The extracts obtained are then stored in a freezer (approximately -18°C) until use.

Quantitative and qualitative characterization of flavonoids extracted: Quantification of total flavonoids in the different fractions of *Solenostemma argel* is performed according to the method of the aluminum trichloride [6]. To one milliliter of each sample was added one ml of the aluminum trichloride solution ($AlCl_3$:2%) in methanol. The mixture was allowed to react for 10 min then the absorbance reading is made at 430 nm. The concentration of flavonoids in the extracts is calculated from the calibration range established with quercetin (1-25 µg/ml) and expressed in milligram of quercetin equivalents per gram of total dry matter (TDM) (mg EQ/g TDM). In this study, the samples were analyzed using commercial plates ready for use, silica gel. Several solvent systems were tested (Table 1).

Antioxidant activity of tested flavonoids was measured using the stable radical chromogen 2,2-diphenyl-1-picrylhydrazide in a photometric assay. The antioxidant activity of tested compounds was determined by measuring the percent of DPPH neutralization by them and compared to the standard antioxidants (gallic acid, quercetin and rutin). It is defined by the index of reducing the percentage scavenging activity (%) RSA (Radical Scavenger Activity) or percent inhibition (%I) or absorbance of the reaction mixture containing the free radical and the sample of the antioxidant is connected with the absorbance of the mixture without any antioxidant (negative control) at a time t:

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

The experimental protocol used is that published by Brand-Williams et al. [7]. The DPPH solution is prepared in methanol. 50 microliter of phenolic extracts or standard solutions are added to 2 ml of DPPH solution. The mixture is left in the dark for 1 hour and discoloration compared to the negative control that contains only the solution of DPPH is measured at 517 nm. Our positive control test is represented by a synthetic gallic acid solution, quercetin and rutin. Concentrations of phenolic extracts of vegetable in the reaction medium, as well as standards used as control in several tests, are 20, 40, 60, 80, 100 µg/ml. The decrease in absorbance is measured by the percent inhibition (%I) and IC_{50} (amount equivalent extract which neutralizes 50% of DPPH). A low IC_{50} corresponds to a high antioxidant activity or anti-radical extract [8,9].

The antibacterial activities of each fraction as well as of the solvent were determined against three bacteria using paper disk plate method on Muller Hinton medium [10,11]. Whatman N° 1 filter paper disks were saturated with the tested materials, and then placed on the agar plate surface which previously inoculated with bacteria (enriched on nutrient broth for 24 hours at 37°C).

The plates were reincubated at 37°C for further 24 hours (bacteria). The disks which had been previously inoculated on the agar plate were observed concerning the zone of growth inhibition adjacent to those disks containing the tested materials to which the bacterium is sensitive.

The development of a zone of growth inhibition of any size around a disk indicated that the organism was susceptible to the examined material. Resistant bacteria grow right up to the margin of the disk [11].

The antibacterial activity of our products is estimated in terms of diameter of the zone of inhibition around the disks containing the flavonoid test products against three pathogens hospital acquired (*Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*) after incubation 24 hours at an ambient adequate temperature of 37°C.

Results and Discussion

Leaves of *S. argel* were successively extracted with methanol/water, then with different solvents which produced 4 fractions.

Quantitative analysis of flavonoids extracts

The results obtained from spectrophotometric determination of amounts representing the total flavonoids (TF) in the various extracts harvested, are determined, and are shown in Table 2 and in histogram (Figure 1).

	TLC on silica gel		
	Solvent systems	**Proportions**	**Bibliography**
System tested	Ethyl acetate/ formic acid/ acetic acid/ H_2O	(100/11/11/27) (v/v/v)	Males et al. [19]
	Ethyl acetate/ methanol/ H_2O	(100/13.5/10) (v/v/v)	Males et al. [19]
	BAW	(60/15/25) (v/v/v)	Diallo et al. [17]
System selected	Ethyl acetate/ methanol/ H_2O	(100/13.5/10) (v/v/v)	Males et al. [19]

Table 1: Different solvent systems used for TLC on silica gel.

Fraction	Amount of TF (mg/g MST)
Diethyl ether	0.47 ± 0.02
Ethyl acetate	1.13 ± 0.03
n-butanol	3.61 ± 0.32
Aqueous	0.76 ± 0.09

Table 2: Results of the determination of total flavonoids obtained by $AlCl_3$ method (mg of quercetin per gram of total dry matter (TDM) of plant material).

This result show that ethyl acetate and butanolic extracts contain the most flavonoic molecules than other extracts (aqueous and diethyl ether) which testifies to the richness of this plant on these phenolic forms.

Quantitative analysis of flavonoids extracts

The qualitative analysis of flavonoids extracts from *Solenostemma argel* revealed that extracts contain a considerable number of components visible on chromatographic profiles reflecting the richness of this plant on flavonoids (Figure 2).

The chromatographic profile of the ether and ethyl acetate extracts appear to be closely related, but it seems like that there are chromatographic bands which are exclusive to ether extract; the compounds having such levels of migration could be responsible for biological activity.

Furthermore, the same level of migration can be obtained by spots corresponding to two different molecular entities, since two phenolic compounds can have the same level of migration therefore the same polarity and even mass, which differentiates them is their appearance under the Ultra-light lamp. It is found that the two compounds are not identical since one absorbs at the wavelength of 254 nm and the other on the contrary appears under a length of 365 nm.

The IC_{50} (the concentration of antioxidant which eliminate 50% of DPPH radicals) was defined for all tested flavonoids and control standard antioxidants (Figure 3).

The negative control corresponds to the same preparation containing DPPH methanolic solution without any antioxidant.

DPHH assay was done in triplicate. The calculation of the error bars in the data set was realized by using Microsoft Office Excel 2007.

The DPPH test is not quantitative, it compares different extracts them according to their ability to scavenge DPPH radical and thus to appreciate the qualitative changes in phenolic compounds.

This figure shows that the references and the extracts of the plant possess important neutralizing capacities; Differences in antioxidant potential between extracts remain. These variations may be due to the composition of the antioxidant molecule extracts. Gallic acid showed better activity compared to other standards, due to the presence of highly reactive (OH) groups, which has been confirmed by many researchers, by way of example [12,13].

Quercetin showed a higher activity than that of its aglycone homologue, rutin. This shows the importance of certain high-reducing phenolic functions which, when involved in glycosidic linkages by engaging these same functions, give a lower activity due to the blocking of the active functions [14].

The best antioxidant activity is attributed to the aqueous extract; this could be explained by the polarity of the solvent which is relatively large allowing the extraction of polar flavonoids, also, the forms having polar functional groups including phenol functional (OH) playing an important role in scavenging of free radicals.

We studied the *in vitro* antibacterial activity of flavonoids isolated from *Solenostemma argel* by using the disk diffusion method on solid agar medium (Muller Hinton). The results are expressed in diameters in millimeters of the inhibition zones formed around the discs loaded in flavonoids (Table 3 and Figure 4).

The solvent tested and taken as a negative control (methanol)

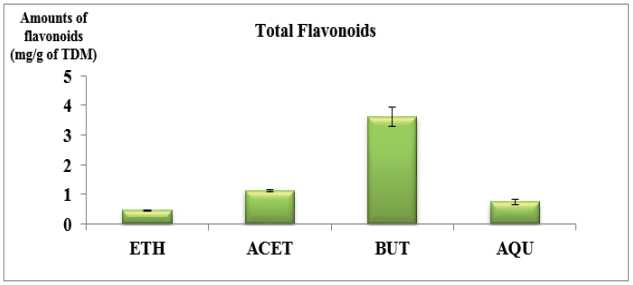

Figure 1: The flavonoid content of *S. argel* fractions. ETH: diethyl ether, ACET: ethyl acetate, BUT: butanol, AQU: aqueous extract. The assay was done in triplicate. The calculation of the error bars in the data set was realized by using Microsoft Office Excel 2007.

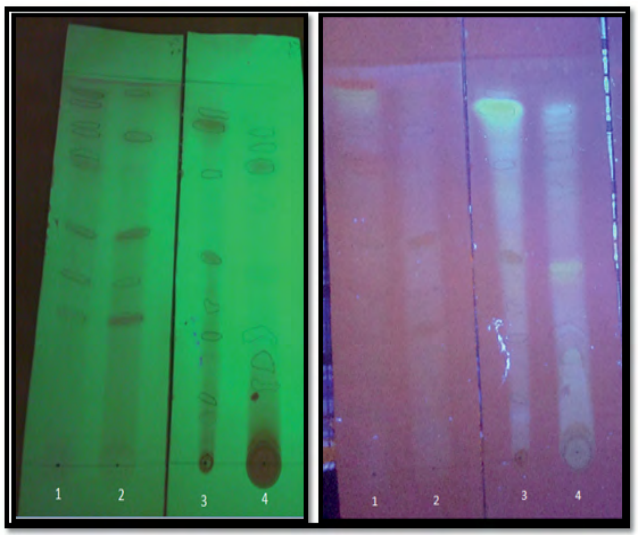

Figure 2: Chromatographic profile of flavonoids extracts obtained from *S. argel* Viewing under a UV lamp at 54 nm (left), at 365 nm (right). 1. Ether extract, 2. Ethyl Acetate, 3. Butanolic fraction, 4. Aqueous extract.

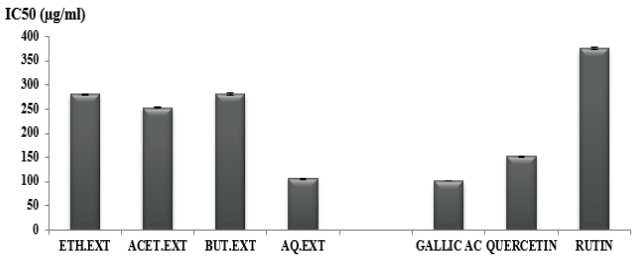

Figure 3: Inhibitory concentrations (IC_{50}) of Solenostemma argel extracts and those of standards.

showed no inhibitory activity against bacterial strains.

The results of the diameters of inhibition's zones showed that *P. aeruginosa* appears sensitive to the flavonoids tested, these flavonoids did not develop zones of inhibition against other strains tested (*Staphylococcus aureus* and *Escherichia coli*).

The sensitivity of *P. aeruginosa* translated the antibacterial action of flavonoids contained in ether extract. In fact, this sensitivity is related

Extract	Diethyl Ether				Ethyl Acetate				Butanol				Water				Rif
Dillutions (%)	100	75	50	25	100	75	50	25	100	75	50	25	100	75	50	25	5 µg
E. coli ATCC25922	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	12 ± 0,568
P. aeruginosa ATCC27853	9	8	8	8	-	-	-	-	-	-	-	-	-	-	-	-	42 ± 3,019
S. aureus ATCC25923	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	12 ± 0,776

(-): No effect/Values represent the mean ± standard deviation (n=3); The negative control corresponds to the solvent alone (methanol) that had no effect against the bacteria tested (data not shown); The concentration used corresponding to the dilution of 100% is 350 µg/ml; The antibiotic used is rifampicin at 5 µg (lot 070531).

Table 3: Results of antibacterial activity of *Solenostemma argel* against three bacterial strains (mm).

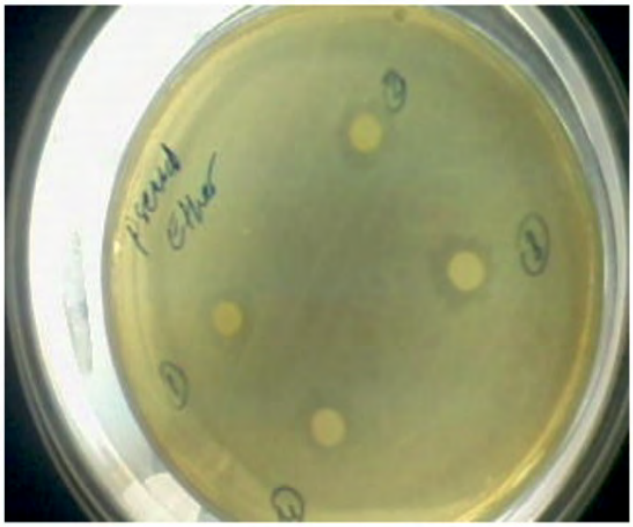

Figure 4: Activity of the ether extract of *Solenostemma argel* against Pseudomonas aeruginosa. Negative control: methanol, Positive control: Rifampicin as antibiotic (Data not shown).

to the number of free hydroxyl which shows that the less hydroxylated flavonoids (hydrophobic molecules) are most active, for example flavonoid that has only one free OH at carbon 5 developing areas inhibition equivalent to 15 mm for *Staphylococcus aureus* and 18 mm for *Escherichia coli*. Cowan [15] assumed that flavonoids without free hydroxyl groups have more antimicrobial activity compared to those that are provided, which leads to an increase in their chemical affinity to membrane lipids, so we can assume that the antimicrobial target of these flavonoids tested is the cytoplasmic membrane. The antibacterial activity of flavonoids can be explained by mechanism of toxicity which is done either by no specific interactions such as the establishment of hydrogen bonds with proteins or cell wall enzymes, chelation metal ions, inhibition of bacterial metabolism, sequestration of substances necessary for the growth of bacteria [16-19].

The difference in activity between these fractions could be explained by the nature of the molecules contained in each of them. Indeed, there are differences in solubility and solvent extraction capacity to phytomolecules. According to Cowan [15], during the liquid-liquid extraction, the phytomolecules are distributed between the solvents according to their polarity and their solubility. It could be deduced that the antibacterial substances contained in *S. argel* in this study, are more soluble in ether than in the other solvents used. Diethyl ether would then concentrate the active ingredient better.

Conclusions

In conclusion, our results demonstrated that the leaves extract of *Solenostemma argel* have potent radical scavenging activities and this effect may be attributed at least in part to the presence of hydroxylated forms (polar).

It emerges from this analysis that each compound acts differently on microorganisms. That is to say, a compound may have a very important action on a seed (the *P. aeruginosa* susceptibility tested flavonoids) or least action, or even zero over another (the resistance of *E. coli* and *S. aureus*). The inhibitory effect against the tested organisms was more effective when using the concentrated extract.

From the present work, it could be recommended that:

Test the different fractions on fungi and other types of bacteria to confirm the hypotheses that the compounds extracted from this plant act on the gram- and gram+ species and try other types of solvents. It would be interesting to use extracts as antibacterial and antifungal agents. And it can be used in the food industry to flavor food and to compact contamination by micro-organisms.

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