Studii şi Cercetări Ştiinţifice 2021, **22** (2), pp. 227 – 234
Chimie şi Inginerie Chimică, Biotehnologii, Industrie Alimentară

Scientific Study & Research
Chemistry & Chemical Engineering, Biotechnology, Food Industry *ISSN 1582-540X*

ORIGINAL RESEARCH PAPER

ANTIOXIDANT ACTIVITY DUAL METHODS OF DIFFERENT POLARITY EXTRACTS OF STEM OF *SOLANUM NIGRUM* L. GROWING IN ALGERIA

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Received: October, 09, 2019
Accepted: June, 24, 2021

Abstract: The purpose of this study is to evaluate the antioxidants properties and phenolic and flavonoid contents of *Solanum nigrum* L. growing in El-Oued region (south east of Algeria). The different polarity extracts chloroform ($CHCl_3$), ethyl acetate (AcOEt) and *n*-butanol (*n*-BuOH) have been prepared from the aqueous ethanol extract of the stem. The antioxidant activity of extracts was determined by DPPH (2, 2-diphenyl-1-picrylhydrazyl), FRAP (Ferric ion reducing antioxidant power) assays. The results showed that $CHCl_3$ extract has the highest level of polyphenol (66.427 ± 0.001 mg gallic acid / g) while, the AcOEt extract has the highest level of flavonoid content (26.450 ± 0.079 mg rutin equivalents / g). In addition, the highest IC50 of DPPH scavenging activity has exhibited the most potent antioxidant capacity assay of $CHCl_3$ (0.231 $mg \cdot mL^{-1}$) compared to the *n*-BuOH and AcOEt extracts. Furthermore, the highest value of FRAP for AcOEt extract was 458.291 ± 0.005. In this study, it has been noted that the potency of the antioxidant activity of DPPH may be identified through different levels of polyphenol. However, the value of FRAP has a relation to the value of flavonoid.

Keywords: *antioxidants properties, DPPH, El-Oued region, flavonoid, FRAP, polyphenol, Solanum nigrum L., stem*

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INTRODUCTION

Medicinal plants have long been used as a cure for many diseases. They are a natural source of chemical molecules such as secondary metabolites that represent very wide variety of organic compounds that enter into the growth and development of plants [1 – 3].
Solanum nigrum L. is also called black night shade [4] or Makoi as commonly called [5], a medicinal plant originated from Eurasia and it was introduced later in America, Australia and South Africa. For a long time, this plant has several benefits in traditional medicine since it is rich in flavonoids, terpenoids, steroids [6, 7]. Moreover, it has various pharmacological properties [8], including antioxidant activity [9]. This research work is concerned with the study of the different phases of aqueous and organic extraction of this plant, that has grown spontaneously in our arid zone (El-Oued), so as to investigate its importance as well as antioxidant activities of bioactive substances of three extracts, *i.e.* ($CHCl_3$, AcOEt, *n*-BuOH) of *Solanum nigrum* L. of *Solanaceae* family.
It has been noted that antioxidant activities of stem of *Solanum nigrum* L. of different polarity extracts have not been extensively reported. However, certain studies have been carried out about another part of plant in terms of their leaves and grain [10, 11].

MATERIALS AND METHODS

Chemical and reagents

Folin Ciocalteu reagent (solution 2 M) was purchased from Sigma Aldrich (Finland), GAE gallic acid (99 %), rutin (97 %), aluminium chloride hexahydrate (97 %), DPPH (2,2-diphenyl-1-picrylhydrazyl) (free radical) (95 %), TPTZ (2,4,6-Tri(2-pyridyl)-1,3,5-triazine) (98 %), Ascorbic acid (99 %).

Instrument

UV-Vis experiments were performed using a UV-1800 UV-VIS spectrophotometer (Shimadzu Scientific Instruments, Kyoto, Japan). For extract evaporation was used Rotavapor mark B.U.C.H.I model R-210, equipped with a top cooler.

Statistical analysis

Results were given as mean ± standard deviation of 3 replicates. The results are expressed as mean values and standard deviation (SD). Descriptive statistical analysis was performed using Microsoft Excel.

Plant Material

The stem of *Solanum nigrum* L. was collected in October 2017 from the area Debila (El-Oued-Algeria).

Before the drying process, the stem was cleaned from insects, coarse parts and gravel. Then, it was divided into small parts to facilitate the drying process. After that, it was stirred twice a day without exposure to the sun for a long time. Once the drying period is over, the crushing of the plant begins so that to prevent it from being rotten [2, 12].

Extraction and isolation

At this point, air-dried stems of *Solanum nigrum* L. were macerated three times during 24 hours at room temperature with both ethanol and distilled water solvents (80 : 20 V / V). After filtration, they were combined and dissolved in distilled water with magnetic stirring and then refrigerated overnight and then, they undergo a second filtration. The resulting solution was successively extracted several times with chloroform, ethyl acetate and *n*-butanol. Furthermore, the organic phases were dried with Na_2SO_4, and were filtered *via* filter paper to obtain the extracts [13].

Phytochemical investigation

Total phenolic content

The method of total phenolic content was described by Singleton-Ross [14] which consists of the use of the Folin-Ciocalteu reagent in order to determine total phenolic content. Briefly, a volume of 100 µL of the extract was mixed with 0.5 mL of freshly diluted 10 flod Folin-Ciocalteu reagent and 2 mL of Na_2CO_3 aqueous (20 % v / w). The reaction mixture was incubated for 30 min. The absorbance of total phenolic was measured at 760 nm against a blank solution. Gallic acid was used as reference and the results were presented as gallic acid equivalents per gram of extract (GAE) / g.

Total flavonoids content

Total flavonoid was determined through the method of Bahorun *and al*. (2003) [15]. A Volume of 1 mL of (2 %) $AlCl_3$ was mixed with 1 mL of the extract. The resulting mixture was incubated for 10 minutes in the dark. The absorbance was measured at 430 nm using UV-VIS spectrophotometer.

Rutin was used as standard. The results were expressed in mg of rutin equivalents of extract (RE) / g.

Antioxidant activity

DPPH scavenging assay

The method of Ohinishi and many others with little amendments [16] was used for the determination of scavenging activity of DPPH radical in the extract solution. A 1 mL of 0.25 mM of DPPH) was prepared in methanol and it was mixed with 1 mL of aqueous extract and the reaction was left in the dark at room temperature for 30 minutes. The absorbance was measured spectrophotometrically at 517 nm, Ascorbic acid being used as antioxidant standard. The results were given as 50 % inhibition concentration (IC50).

The inhibition power is expressed in % and determined by applying the following: (Equation 1):

$$I\ \% = [(A_0 - A_S) / A_0] * 100 \quad (1)$$

where: A_0 - absorbance of the control;
A_S - absorbance of the sample.

Ferric antioxidant power (FRAP) assay

The reducing power was determined by using FRAP assay which was performed based on the procedure described by Benzie *et al*. and strain [17]. 100 µL of the sample extract was added to 300 µL distilled water then 3 mL of the FRAP reagent (25 mL of 0.3 M acetate buffer, *p*H = 3.6, plus 2.5 mL of 10 mM TPTZ in 40 mM HCl and 2.5 mL of 20 mM $FeCl_3$) was incubated for 30 min. The absorption of the reaction was mixture at 593 nm. $FeSO_4$ was used as standard with concentration varying. The results were expressed as mg Fe (II) /g of extract.

RESULTS AND DISCUSSION

First, the determination of the total bioactive compounds is presented. Then, the test of antioxidant activities (DPPH, FRAP) is reported.

Determination of total bioactive compounds

Total phenolic content (TPC) in various extracts were demonstrated in terms of gallic acid equivalent using the standard curve (Equation 2):

$$y = 3.7143x - 0.0914,\ R^2 = 0.9951 \quad (2)$$

TPC in various extracts of *Solanum nigrum* L. stem showed different results ranged from 21.186 to 66.427 mg GAE / g. Moreover, chloroform had the highest phenolic content (66.427 mg GAE / g) for stem extracts (Table 1 and Figure 1).

Table 1. *Total phenolic content in various extracts of Solanum nigrum* L. *stem*

EXTRACT	$CHCl_3$ extract	AcOEt extract	*n*-BuOH extract
Polyphenol content [mg GAE / g]	66.427 ± 0.001	63.735 ± 0.001	21.186 ± 0.002

Total flavonoids content (TFC) in the various extracts was demonstrated in terms of Rutin equivalent using the standard curve (Equation 3):

$$y = 11.787x + 0.0991,\ R^2 = 0.9912 \quad (3)$$

The amount of TFC from stem ranged from 3.322 to 23.591 mg RE / g of extra (Table 2 and Figure 1). The overall results indicate that the highest amount of TFC was obtained from stem part was for ethyl acetate extract (23.591 mg RE / g).

Table 2. *Total flavonoid content in various extracts of Solanum nigrum* L. *stem*

EXTRACT	$CHCl_3$ extract	AcOEt extract	*n*-BuOH extract
Flavonoid content [mg RE / g]	12.165 ± 0.009	23.591 ± 0.023	3.322 ± 0.009

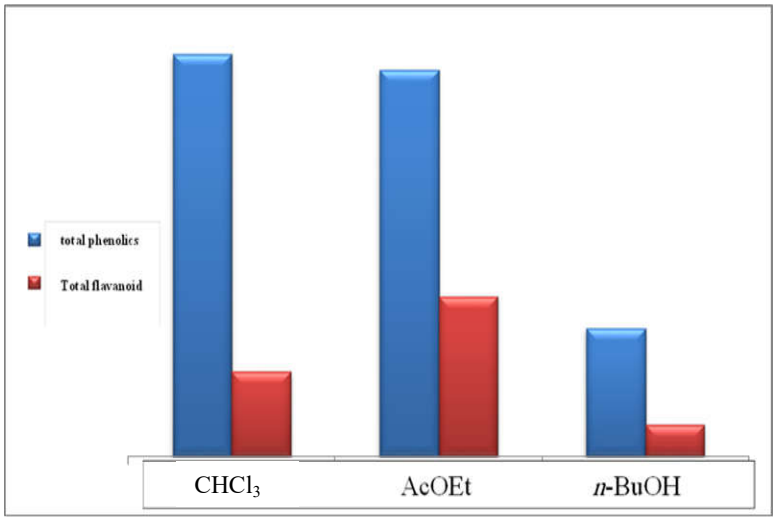

Figure 1. *Total phenolic and total flavonoid contents in the three extracts of Solanum nigrum* L.

According to the results, solvents variation leads to the modification of total phenolic and flavonoid contents. It has been observed that the chloroform extract contains the largest quantity of phenols, and the acetate extract contains a large amount of flavonoids compared to other extracts. This is due to the difference in solubility and polarity of the compounds in the solvents. This means that chloroform has the greatest capacity to dissolve the existing phenols in the stem of the plant. Acetate also has the highest ability to dissolve flavonoids in the same part of the plant.

In another study about stem of *Solanum nigrum* L. [18], the amount of phenols was estimated at (0.53 mg GAE / g) prepared in ethanolic extract. The amount of flavonoids was also found in the same study (1.32 mg QE / g) was also obtained from ethanolic extract.

In comparison to what have obtained, it has been noted that the amount of phenols and flavonoids obtained in this study is higher than this study due to different solvents and extraction method.

It is worth mentioning that there have been fewer previous studies concerning the antioxidant activity of the three different polarity extracts (chloroform, ethyl acetate and *n*-butanol) from *Solanum nigrum* L.

Antioxidant activity

Two methods were adopted to evaluate the antioxidant properties of the extract; DPPH free radical scavenging activity which measures the ability of electron transfer or give hydrogen atom and the FRAP method which also measures electron transferring of the antioxidant (Table 3).

Table 3. *Antioxydants activity of Solanum nigrum extracts of by DPPH assay*

EXTRACT	**$CHCl_3$ extract**	**AcOEt extract**	***n*-BuOH extract**	**Ascorbic acid**
IC50 [mg·mL^{-1}]	0.231	0.234	0.390	0.006

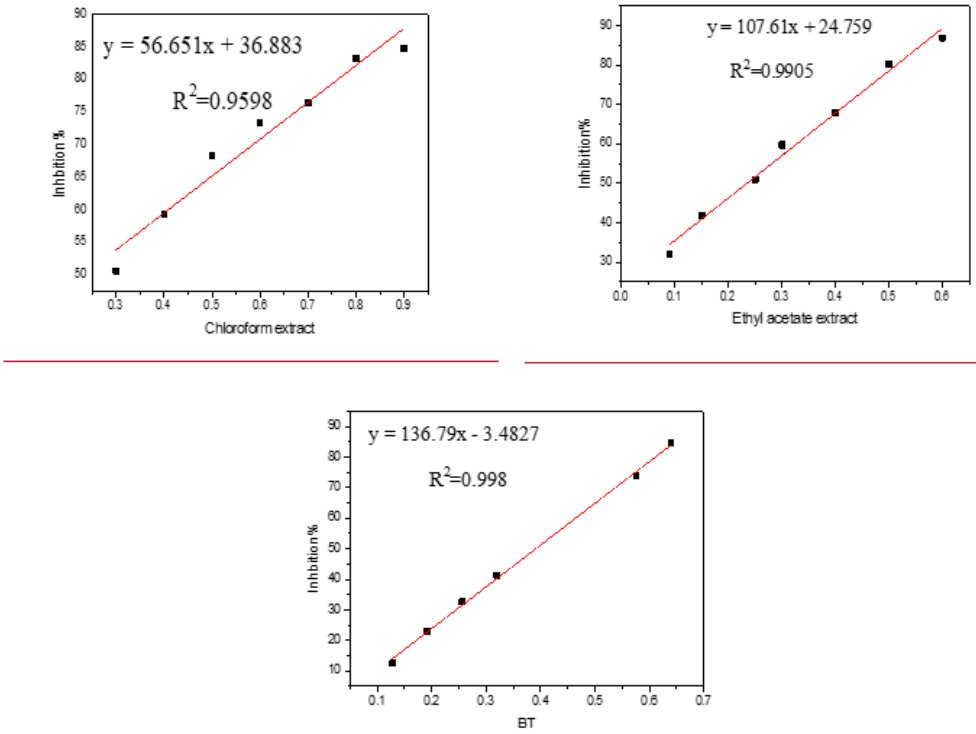

Figure 2. *Curves inhibition of three extract of DPPH assay*

The free radical scavenging activity of the three different extracts from the stem of *Solanum nigrum* L. is expressed in terms of percentage of inhibition (%) and IC50 values (mg·mL^{-1}) (Table 3 and Figure 2). Parallel to examination of the antioxidant activity of these plant extracts, the value of standard compound was obtained and compared to the value of the antioxidant activity. The examination of antioxidant activities of the extracts from stem has exhibited different values which varied from 12.43 to 84.77 %. Furthermore, the largest capacity to neutralize DPPH radicals was found for chloroform extract which neutralized 50 % of free radicals at the concentration of 0.231 mg·mL^{-1}.

A moderate and almost equal activity was found for AcOEt extract 0.234 mg·mL^{-1} than *n*-BuOH extract which had the lowest activity. In comparison to IC50 value of ascorbic acid, chloroform extract has shown the strongest capacity for neutralization of DPPH radicals.

In a previous study [9] of the DPPH inhibition test, the activity of the ethanolic extract from the stems of *Solanum nigrum* L. has given a lower activity than that of ascorbic acid. Then, the value of IC50 in this study was estimated at 301.99 µg·mL^{-1}, which is higher than the values obtained in our extracts, which provided better results.

The results of FRAP assays in different extracts showed values ranged from 198.4 to 458.29 mg $FeSO_4$ / g (Table 4). The ethyl acetate extract manifested the highest activity (458.29 ± 0.005), followed by *n*-butanol (348.89 ± 0.004) and chloroform extract (198.41 ± 0.003).

Table 4. *Antioxydants activity of extracts of Solanum nigrum* L. *by FRAP assay*

EXTRACT	**$CHCl_3$ extract**	**AcOEt extract**	***n*-BuOH extract**
FRAP [mg $FeSO_4$ / g]	198.41 ± 0.003	458.29 ± 0.005	348.89 ± 0.004

An extensive literature review has shown that there was no previous study which dealt with antioxidant activity of FRAP assay of stem from *Solunum nigrum* L.
These results indicate the amount of polyphenols that plays a role as antioxidants and natural reducing [18] is proportionate with the antioxidant activity by DPPH assay. This could be explained by the principle of reaction [19]. The DPPH radical reducing large amounts of molecules consist of hydrogen donor. These molecules are considered as phenolic compounds found within chloroform extract that has lower polarity containing methoxyl group more than carboxylic one in its chemical structure [20].
As revealed by the FRAP method, which measures the proportion of antioxidants reduced by complex TPTZ [21, 22], the amount of antioxidants found abundantly in the acetate extract corresponds to that of flavonoids present in the same polar extract, which is characterized by the presence of groups of carboxylic in its chemical structure [21].

CONCLUSION

In conclusion, it is safe to say that the use different polarity of solvents made phenolic compounds dissolve due to their chemical properties and their structure. The differing principle of the DPPH and FRAP methods to measure antioxidant activity explains the difference in obtained yield.
The study concludes that there was a relationship between DPPH-measured antioxidants, polyphenols, FRAP-measured antioxidants and flavonoids.
This study has supported previous research on the *Solanum nigrum* L. plant, as well as the studied region in order to contain stem part on antioxidants and a significant amount of active compounds.

ACKNOWLEDGEMENT

We are grateful to the support that was offered from Laboratory of *Valorization and Technology of Saharan Resources* (VTRS). We also thank our University Kasdi Merbah Ouargla.

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