Journal of Applied Pharmaceutical Science Vol. 7 (04), pp. 192-198, April, 2017
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2017.70428
ISSN 2231-3354

Phytochemical screening and evaluation of antioxidant and antibacterial activities of seeds and pods extracts of *Calycotome villosa* subsp. Intermedia

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ARTICLE INFO

Article history:
Received on: 28/01/2017
Accepted on: 26/03/2017
Available online: 30/04/2017

Key words:
Calycotome villosa subsp. intermedia, antioxidant activity, total flavonoids contents, total phenolic contents, antibacterial activity.

ABSTRACT

In the present study, antioxidant potential of the methanol and the ethyl acetate extracts of the seeds and pods of *Calycotome villosa subsp. intermedia* were evaluated by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging assay, reducing power and total antioxidant capacity. In DPPH scavenging assay, the IC_{50} value of the methanolic extract of seeds was found to be 0.20 mg/mL, the standard reference value for butylatedhydroxytoluene (BHT) is 0.19 mg/mL. Total antioxidant activity was also found to increase in a dose dependent manner. Moreover, *Calycotome villosa* extract showed strong reducing power. The total phenolic content in the extracts was determined using Folin-Ciocalteu reagent and their amounts ranged between 173.37 ± 0.02 to 332.23 ± 0.01 mg GA (Gallic Acid)/g of dry extract. The concentrations of flavonoids in the extracts varied from 5.02 ± 0.03 to 66.45 ± 0.01 Qu (Quercetin)/g of dry extract. Ethyl acetate extract of seeds of *Calycotome villosa* showed the highest phenolic, flavonoids concentration and the strongest antioxidant activity. The high contents of phenolic compounds indicated that these compounds contribute to the antioxidant activity. Furthermore, all extracts showed good antibacterial activity against *Escherichia coli*. *Calycotome villosa subsp. intermedia* can be regarded as promising candidate for natural plant sources of antioxidants with high value.

INTRODUCTION

Antioxidant research is an important topic in the medical field as well as in the food industry. Many plants, particularly medicinal ones, have been extensively studied for their antioxidant activity in recent decades. Antioxidants from aromatic, spicy, medicinal, and other plants were studied to develop natural antioxidant formulations for food, cosmetic, and other applications (Miliauskas *et al*., 2004). It is believed that an increased intake of food rich in natural antioxidants is associated with lower risks of degenerative diseases, particularly cardiovascular diseases and cancer (Perez-Jimenez *et al*., 2008). There are three major classes of plant chemicals: terpenoids, phenolic, and alkaloids (Harborne, 1999). Among these three groups, phenolic compounds are the most important for dietary applications and the most extensively researched (King and Young, 1999). Phenolic compounds include phenolic acids (hydroxybenzoic and hydroxycinnamic acids), polyphenols (hydrolyzable and condensed tannins), and flavonoids. These compounds can scavenge excess free radicals and effectively reduce oxidative stress, which protect plants, fruits, and vegetables and protect DNA, protein and lipids in the human body from oxidative damage. They have been used as antioxidants by humans, thus preventing diseases from being induced. Finding new and safe antioxidants from natural sources is of great interest for applications as natural antioxidants, functional foods, and neutraceuticals.

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Phytochemical screening is one of the methods that have been used to explore antioxidant compounds in plants. *Calycotome villosa*, a genus of flowering plants in the legume family (Leguminosae), grows mostly in cool places, and is very common in the Mediterranean area (Gibbs, 1968; Tutin, 1972). However, *Calycotome villosa* (Poiret) Link subsp. *intermedia* (C. Presl) Quezel and Santa, a 50-150 cm spiny shrub, grows especially in the north of Africa and Spain (Greuter *et al*., 1989). In Morocco this plant settles in degraded areas, forming an abundant shrub stratum (Bonin, 1994). A survey of the bibliography showed us no uses in the Moroccan folk medicine are known for this species. Medicinal uses have been reported only for *Calycotome villosa* (Poiret) Link as antitumoral agent (Hartwell, 1982) and for the treatment of furuncle, cutaneous abscess and chilblain in the Sicilian folk medicine (Lentini *et al*., 1993). In previous phytochemical studies of *Calycotome villosa* subsp. *intermedia*, we have reported the isolation and characterization of flavone glucosides from the leaves and flowers (El Antri *et al*., 2004a), flavonols from the seeds (El Antri *et al*., 2010), tetrahydroisoquinoline alkaloids, isoquinoline-N-oxide and dihydroisoquinoline-N-oxide alkaloids, and a paraben derivative from the seeds (El Antri *et al*., 2004b, 2004c, Elkhamlichi *et al*., 2014). The aims of this study were to screen for phytochemical constituents, determine the total phenolic and flavonoid contents, and evaluate *in vitro* antioxidant activity of seeds and pods extracts of *Calycotome villosa subsp. intermedia*. Three different antioxidant test systems were performed: DPPH, reducing power and total antioxidant capacity assays. Antibacterial proprieties were also examined.

MATERIAL AND METHODS

Plant material

Seeds and pods of *Calycotome villosa subsp. intermedia* were collected from the aerial part of the plant in June 2011 and again in June 2012 from area of Sefrou (Morocco). The plant was identified by Dr. Abdeslam Ennabili (Sidi Mohammed Ben Abdellah University, Fez, Morocco). A voucher specimen (n° EN008) has been deposited at the herbarium of the ''Institut National des Plantes Médicinales et Aromatiques'', Sidi Mohammed Ben Abdellah University, Taounate, Morocco.

Preparation of the extracts

The seeds and pods were used just after harvest and they were crushed with a grinder. The powdered seeds (154.13 g) and pods (68.16 g) of *Calycotome villosa subsp. intermedia* were first extracted with hexane for 24 h to remove chlorophylls and fats, then with methanol for 48 h using a Soxhlet apparatus. The methanolic solution was evaporated to dryness and the resulting crude seeds extract (18.3 g) and pods extract (13.08 g) was dissolved in a 5% hydrochloric acid solution. The residue was solubilized in methanol; the methanol residue which is not soluble in methanol was dissolved in ethyl acetate and evaporated under reduced pressure by a rotary evaporator to give respectively 3.93 g of the crude extract of seeds and 1.87 g of the crude extract of pods.

Phytochemical screening

Phytochemical screening to detect the presence of bioactive agents was performed by standard procedures (De *et al*., 2010). After the addition of specific reagents to the solution, the tests were detected by visual observation of color change or by precipitate formation.

Total phenolic contents

The total phenolic contents were determined spectrophotometrically using the Folin-Ciocalteu reagent. This reagent based on the Slinkard and Singleton method (Slinkard and Singleton, 1977) and the early work of Singleton and Rossi (Singleton and Rossi, 1965) is a colorimetric oxidation/reduction method for phenolic compounds. Briefly, extracts were reacted with Folin-Ciocalteu reagent and then neutralized with sodium carbonate solution (25%). After 2 h, the absorbance of the resulting solution was measured at 765 nm. The concentrations of phenolic compounds were calculated according to the following equation that was obtained from the standard as gallic acid graph:

Absorbance = 0.0007 gallic acid (µg) + 0.0642 (R^2 = 0.9964)

All tests were carried out in triplicate and the results are given as gallic acid equivalents (GAE) per g of dry extract.

Total flavonoid contents

Total flavonoid contents were determined using the Dowd method as adapted by Arvouet-Grand (Arvouet-Grand *et al*., 1994), 1 mL of 2% aluminium trichloride ($AlCl_3$) in methanol was mixed with the same volume of the methanolic extracts (2000 µg). Absorption readings at 415 nm were taken after 10 min against a blank sample consisting of a 1 mL extract solution with 1 mL methanol without $AlCl_3$. The concentrations of flavonoid compounds were calculated according to the following equation that was obtained from the standard quercetin graph:

Absorbance = 0.0333 quercetin (µg) + 0.0231 (R^2 = 0.9961)

Antioxidant studies

In the present study, three commonly used antioxidant evaluation methods such as DPPH radical scavenging activity, reducing power assay and phosphomolybdenum method were chosen to determine the antioxidant potential of seeds and pods of *Calycotome villosa subsp. Intermedia.*

Determination of free radical scavenging activity by DPPH method

The DPPH (1,1-diphenyl-2-picrylhydrazyl) assay is one of the most commonly employed methods because, in general terms, it is simple, efficient and inexpensive. The original method was developed by Blois (Blois, 1958) and, with the modifications introduced by Brand-Williams, Cuvelier, and Berset (Brand-Williams, 1995), it is widely used as a reference point (Bondet *et al*., 1997; Chen *et al*., 2013). The results are normally expressed as

Efficient Concentration (EC_{50}) otherwise called the IC_{50} value, which is defined as the amount of sample necessary to decrease the initial DPPH concentration by 50%. The parameter IC_{50} was introduced by Brand-Williams (Brand-Williams, 1995), Molyneux (Molyneux, 2004), Kedare and Singh (Kedare and Singh, 2011), and it is very useful for comparing results because it is independent of the sample concentration. Many authors use the antiradical power (ARP) parameter, which is defined as the reciprocal of IC_{50}: $ARP = 1/IC_{50}$. The DPPH assay was employed to test the antioxidant potential of the ethyl acetate and the methanolic extracts of the seeds and pods of *Calycotome villosa subsp. intermedia*. Briefly, 100 µL of various concentrations of the extract in methanol was added to 10 mL of a methanol solution of DPPH (1.014×10^{-2} M). The mixture was vigorously shaken and then allowed to stand at room temperature for 30 min in the dark. The absorbance of the mixture was measured at 517 nm by using a double-beam UV-visible Camspec M550 spectrophotometer. A mixture of 100 µL of methanol and 10 mL of methanol solution of DPPH was used as the control. The scavenging activity on the DPPH radical was expressed as inhibition percentage using the following equation:

% Inhibition= $[(Abs_{control} - Abs_{sample}) / Abs_{control}] \times 100$ (Blois, 1958)

Butylatedhydroxytoluene (BHT) was used as positive control. The tests were carried out in triplicate. The extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph of inhibition percentage plotted against extract concentration (4.0, 2.0, 1.0, 0.5 and 0.25 mg/L) (Viturro, *et al*., 1999).

Total antioxidant capacity (TAC)

The total antioxidant capacity of the methanol extract and the ethyl acetate were evaluated by the phosphomolybdenum method according to the procedure described by Prieto (Prieto *et al*., 1999). The assay is based on the reduction of Mo(VI) to Mo(V) by the extract and subsequent formation of green phosphate/Mo(V) complex at acid pH. A 0.1 mL extract was combined with 1 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The test-tubes containing the reaction solution were incubated at 95ºC for 90 min. Then the absorbance of the solution was measured at 695 nm using a double-beam UV-visible Camspec M550 spectrophotometer against blank after cooling to room temperature. Methanol (0.1 mL) in the place of extract was used as the blank. The antioxidant activity is expressed as the number of gram equivalent of ascorbic acid. The calibration curve was prepared by mixing ascorbic (100, 50, 25, 12.5 and 6.25 µg/mL) with methanol. Methanol (0.1 mL) in the place of extract is used as the blank. The antioxidant activity is expressed as the number of equivalents of ascorbic acid.

Ferric reducing antioxidant power (FRAP)

The ferric reducing antioxidant power was determined according to the method previously described by Oyaizu (Oyaizu, 1986). According to this method, the reduction of Fe^{3+} to Fe^{2+} is determined by measuring the absorbance of Perl's Prussian blue complex. Briefly, different concentrations of extracts (5-200 µg) in 1 mL of distilled water were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide $[K_3Fe(CN)_6]$ (2.5 mL, 1 %).

The mixture was incubated at 50°C for 20 min. An aliquot (2.5 mL) of trichloroacetic acid (10 %) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The supernatant (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1 %) and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. Ascorbic acid was used as the reference.

Determination of antibacterial activity

Test micro-organisms

A total of four bacterial species were tested including *Pseudomonas aeroginosa* ATCC 27853, *Escherichia coli* ATCC 25922 (Gram-negative), *Bacillus subtilis* ILP 14283 and *Staphylococcus aureus* (Gram-positive) ATCC 25922 have been used. The strains were cultured on nutrient agar and incubated at 37°C for 24 h and were then maintained in their appropriate agar medium at 4°C throughout the study and used as stock cultures.

Disc diffusion assay

The antibacterial activity of the methanol extracts and ethyl acetate extracts was examined by disk-diffusion method (NCCLS, 2012) with some modifications. Briefly, bacterial strains were cultured overnight at 37°C on Luria-Bertani broth, and then inoculum consisting of 0.5 McFarland was prepared in physiologic saline. Bacterial inoculum (100 µL) was inoculated in Petri dishes containing a sterile Luria-Bertani Agar medium. Sterile filter paper discs (5 mm diameter) were deposited on medium and impregnated with 10 µL of extract solution (20 mg/mL of DMSO to 2%).

The plates were inverted and incubated for 20 h at 37°C. The control was performed with discs containing 10 µL of DMSO to 2%. Each experiment was performed in duplicate. Microbial inhibition was determined by measuring the diameter of the clear zone of inhibition of growth around each disc and recorded as diameter of inhibition zone in millimeter. The scale of measurement was the following (disc diameter included): ≥ 20 mm: zone of inhibition is strongly inhibitory; < 20-12 mm: zone of inhibition is moderately/mildly inhibitory; and < 12 mm is no inhibitory (Espina *et al*., 2011).

Statistical analysis

All analyses were done at least in triplicate, and these values were then presented as average values along with their standard derivations. Statistical comparisons were performed with one way analysis of variance, and p values < 0.05 were regarded as significant. Correlation coefficients (R) to determine the relationship among TAC assay, TPC and TFC were calculated using MS Excel software (CORREL statistical function).

RESULTS AND DISCUSSION

Phytochemical screening

The results of phytochemical screening of seeds and pods for the methanol and ethyl acetate extract of *Calycotome villosa subsp. intermedia* are given in the Table 1. The cyanidin test for flavonoids showed the presence of flavones in methanolic extract and the flavonols in ethyl acetate extract which is confirmed by a previous study, two flavonols no glycosylated were isolated from the seeds (El Antri *et al*., 2010). In pods extracts, phytochemical analysis revealed the presence of flavones from the methanolic and ethyl acetate extracts, the tannins with high presence in ethyl acetate. The methanolic and ethyl acetate extracts doesn't contain the alkaloids which were dissolved in a 5% hydrochloric acid solution. The saponins were not detected in these extracts.

Table 1: Phytochemicals detected in extracts of *C. villosa* seeds and pods.

Categories	Phytochemicals	Methanol	Ethyl acetate
Pods	Alkaloids	-	-
	Flavonoids	+	+
	Tannins	+	+
	Saponins	-	-
Seeds	Alkaloids	-	-
	Flavonoids	+	+
	Tannins	+	+
	Saponins	-	-

Key: + for present; − for absent.

Total phenolic contents

Total phenolic and flavonoid contents and antioxidant activity *in vitro* were determined for methanol and ethyl acetate extracts of seeds and pods of *Calycotome villosa subsp. intermedia*. The results of the total phenolic content determination of the examined plant extract are presented in Table 2. The content of total phenols in different extracts, expressed as gallic acid equivalents (GA) per gram of dry extract, ranged between 173.37 ± 0.02 to 332.23 ± 0.01 mg GA/g. The highest phenolic content was found in methanol extracts (with 2 mg/mL extract concentration). The phenolic compounds are generally more soluble in polar solvents, in this research the logic was respected since the crude extract; the methanolic extracts of seeds and pods have the highest phenolic content than ethyl acetate extract.

Table 2: Total phenolic contents.

Categories	Sample	Total phenolic content (mg GAE /g DE)
Seeds	MeOH	332.23 ± 0.01
	EtOAc	243.94 ± 0.03
Pods	MeOH	285.37 ± 0.01
	EtOAc	173.37 ± 0.02

Total flavonoids contents

In both categories, the total flavonoids contents in seeds were significantly higher than in pods. Comparing the extracts of the same part of plant, it was found that methanolic extracts had higher contents of total flavonoids than ethyl acetate extracts. The result of total flavonoids content is summarized in Table 3. The content of flavonoids (mg/g), in quercetin equivalents (Qu E), varied from 5.02 ± 0.03 to 66.45 ± 0.01. Of the two extracts of each vegetable, the extracts of seeds had the highest flavonoid contents, with that of methanolic extract being the highest (66.45 ± 0.01).The total content of flavonoids and phenolics are influenced by the interaction between parts of plants (Ghasemzadeh *et al*., 2010).

Table 3: Total flavonoids contents.

Categories	Sample	Total flavonoids contents (mg QuE / g DE)
Pods	MeOH	22.20 ± 0.02
	EtOAc	5.02 ± 0.03
Seeds	MeOH	66.45 ± 0.01
	EtOAc	43.4 ± 0.02

Antioxidant studies

Dietary antioxidants have a dual role: they can prevent food oxidation, in particular lipid oxidation, and at the same time, increase the antioxidant intake from diet. In organism, these exogenous antioxidants can manifest a wide variety of actions, including inhibition of oxidizing enzymes, chelation of transition metals, transfer of hydrogen or a single electron to radicals, singlet oxygen deactivation, or enzymatic detoxification of reactive oxygen species (Morales-González, 2013). Common result of these actions is the protection against degenerative diseases, for example, atherosclerosis, cancer, diabetes, rheumatoid arthritis and inflammatory diseases, which are caused by the increased level of reactive radical species (Prior *et al*., 2005). Therefore, in order to extensively characterize antioxidant potential of the *C. villosa subsp. intermedia* extracts examined, different antioxidant assays were applied. Examined seeds and pods extracts demonstrated antioxidant potency regarding the reactions based on single-proton/electron transfer (DPPH$^{\bullet}$, FRAP and TAC assays). However, these activities were manifested at moderate intensity in comparison to standard antioxidants butylatedhydroxytoluene (BHT), gallic acid (GA), ascorbic acid (AA) and tannic acid (TA).

Determination of free radical scavenging activity by DPPH method

The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity is given in Figures 1 and 2. This activity was increased by increasing the concentration of the sample extract at 518 nm. DPPH antioxidant assay is based on the ability of a potential antioxidant to scavenge the stable radical of 1,1-diphenyl-2-picrylhydrazyl (DPPH), a stable free radical contains an odd electron, which is responsible for a strong absorption band in the range of 515-520 nm. In the presence of antioxidant compounds, DPPH can accept an electron or a hydrogen atom from the antioxidant scavenger molecule, to be converted to a more stable DPPH molecule. As the reduced form of DPPH is pale yellow, the greater the free radical scavenging capacity of an antioxidant compounds, is identified by the more reduction of DPPH and the less purple color there is in the sample. The assessment of antioxidant activity showed that the examined seeds were able to scavenge this radical (Figure 1).

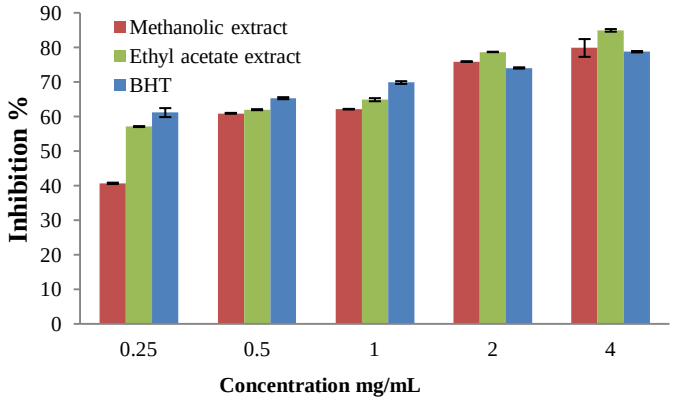

Fig. 1: Antioxidant activities of methanol and ethyl acetate extracts from *Calycotome villosa* seeds measured by DPPH method. BHT was used as reference antioxidant.

Ethyl acetate extract of seeds showed a high antioxidant activity, being able to scavenge more than 80% of the DPPH radical at concentration of 4 mg/mL which displayed the highest activity than methanolic extract (IC_{50} values were: 0.2, 0.34 mg/mL, respectively). A higher DPPH radical-scavenging activity is associated with a lower IC_{50} value. Different extracts of pods are shown in Figure 2.

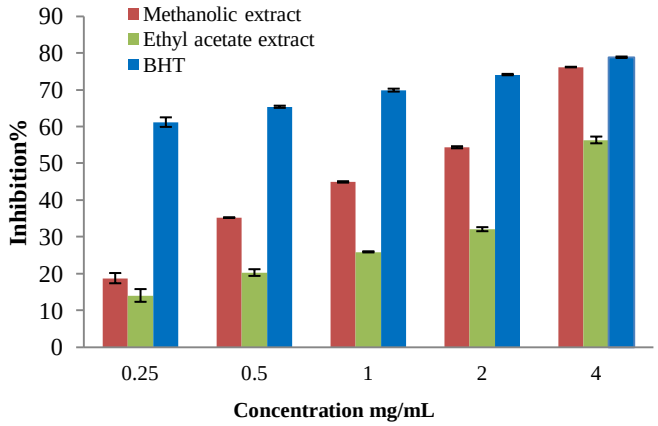

Fig. 2: Antioxidant activities of methanol and ethyl acetate extracts from *Calycotome villosa* pods measured by DPPH method. BHT was used as reference antioxidant.

Ethyl acetate extract showed very weak DPPH radical scavenging activity, when compared to those reported for methanol extract which were active and able to scavenge 76.15 ± 0.04 % of the DPPH radical at concentration of 4 mg/mL. In the same context, the results of DPPH tests for pods extracts showed that IC_{50} values obtained for the samples submitted to DPPH assay were in the ranged from 1.53 to 3.5 mg/mL. As mentioned above, this plant contains a number of compounds such as flavones glycosides (El Antri *et al.*, 2004a), flavonols (El Antri *et al.*, 2010), and tannins which were responsible for a higher DPPH radical scavenging activity. In this respect, our results indicate that the seeds and pods of *C. villosa subsp. intermedia* showed high free radical scavenging activity proposing that these parts of plant may be used as a cheap source for natural antioxidants.

Total antioxidant capacity (TAC)

In the phosphomolybdenum assay, which is a quantitative method to evaluate the antioxidant capacity (Arabshahi-Delouee and Urooj, 2007), all extracts exhibited different degrees of activity as shown in Figures 3 and 4. All crude extracts showed increasing antioxidant activity with increasing concentration. It was, however, observed that the ethyl acetate extract of the seeds possesses significant total antioxidant capacity equivalent to 157.6 mg/g ascorbic acid at higher concentration (100 µg/mL), and followed by methanolic extract. Gallic acid, as positive control, was found to be less efficiency (130.88 mg/g ascorbic acid equivalents) at the same concentration. The crude extracts of pods showed the lowest antioxidant activity.

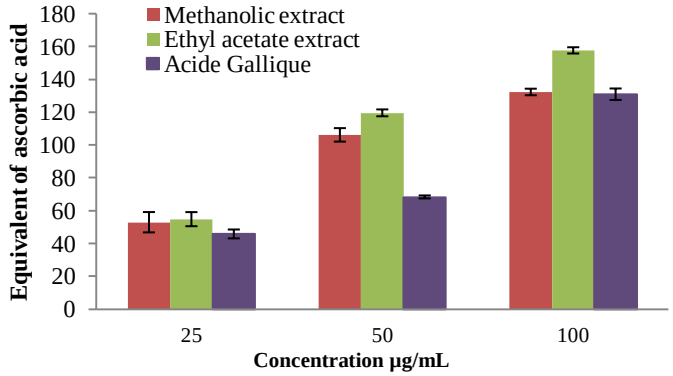

Fig. 3: Total antioxidant activity (TAC) of methanol and ethyl acetate extracts from *Calycotome villosa* seeds.

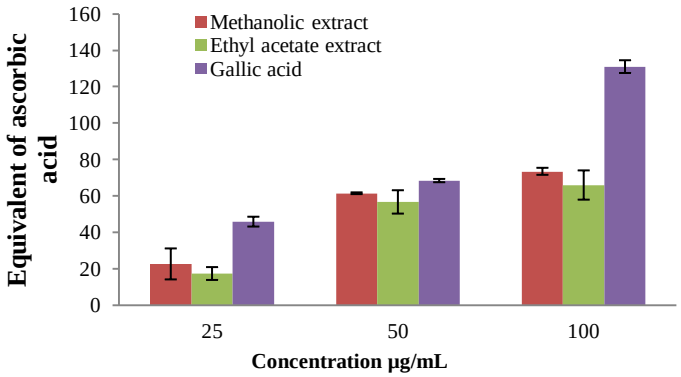

Fig. 4: Total antioxidant activity (TAC) of methanol and ethyl acetate extracts from *Calycotome villosa* pods.

Ferric reducing antioxidant power (FRAP)

The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Osawa, 1994). For the measurements of the reductive ability, it has been found that the Fe^{3+}–Fe^{2+} transformation occurred in the presence of extract samples which were postulated previously by Oyaizu (Oyaizu, 1986). Earlier authors (Tanaka *et al.*, 1988) have observed a direct correlation between antioxidant activity and reducing power of certain plant extracts. The reducing properties are generally associated with the presence of reductones (Duh *et al.*, 1999),

which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom (Gordon, 1990). Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation. Figure 5 shows the reductive capabilities of the plant extracts compared to ascorbic acid and to tannic acid.

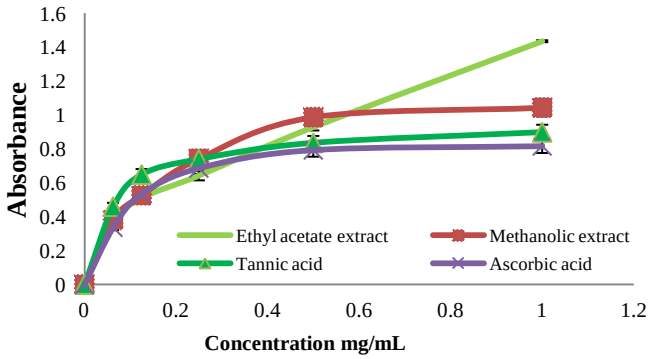

Fig. 5: Reducing power of the crude seeds extracts. Values are the average of duplicate experiments and represented as mean ± standard deviation.

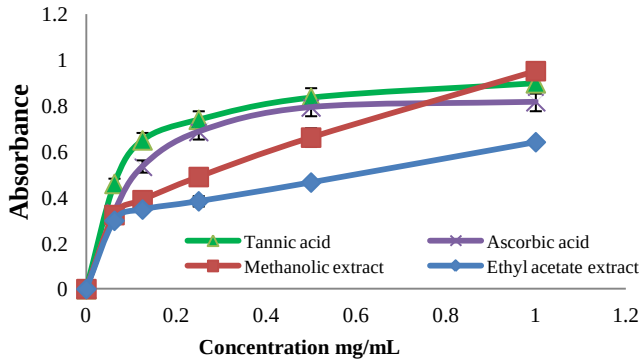

Fig. 6: Reducing power of the crude pods extract. Values are the average of duplicate experiments and represented as mean ± standard deviation.

The reducing power of the seeds extracts of *Calycotome villosa subsp. intermedia* was found remarkable indeed the ethyl acetate extract shows a high reducing ability relative to the methanolic extract which is better than ascorbic acid and tannic acid at concentration of 0.32 mg/mL. Figure 6 shows the reducing power of the methanolic extract and ethyl acetate extract of the pods which were less than those of ascorbic acid and tannic acid except for the methanolic extract at concentration of 0.79 mg/mL. The activity was found in the order, methanolic extract > ethyl acetate extract, which was in correlation with the presence of total phenolics and flavonoids content in the respective extracts. The reducing power of the extract was observed to rise as the concentration of the extract was gradually increased. These results suggest that all the extract possess phenols or some other compounds with hydrogen donating ability.

Antibacterial activity

The results of the antibacterial activity were presented in Table 4. The negative control used (DMSO 2%) did not exert any inhibition on the strains tested. In the present study, ethyl acetate and methanol extracts of seeds and pods exhibit remarkable antibacterial activity against the strain (*Escherichia coli*). However, gram positive strains and *P. aeroginosa* tested were resistant to all extracts. Ethyl acetate extract of seeds showed weak inhibition against *Bacillus subtilis*, whereas its activity toward the other two tested organisms was found to be absent. It is worth noting that all of the extracts showed greater potent antibacterial activity gram negative bacteria, especially *Escherchia coli* (inhibition zone: 50 mm).

Table 4: The growth-inhibitory diameters (mm) of methanol and ethyl acetate extracts of seeds and pods against the tested bacteria.

Bacterial strains	Pods		Seeds	
	Ethyl acetate extract	**Methanol extract**	**Ethyl acetate extract**	**Methanol extract**
Escherichia coli ATCC 25922	Total inhibition	Total inhibition	Total inhibition	Total inhibition
Pseudomonas aeroginosa ATCC 27853	-	-	-	-
Bacillus subtilis ILP 14283	-	-	8 mm	-
Staphylococcus aureus ATCC 25922	-	-	-	-

Key: - = no antibacterial activity detected.

CONCLUSION

The results of antioxidant evaluation based on the three models (DPPH, TAC & FRAP) used in this study revealed that ethyl acetate and methanol extract of *Calycotome villosa subsp. intermedia* seeds possess interesting antioxidant activity followed by methanol extract of pods. Based on the distribution of metabolites quantified in each of the parts, it can be seen that the values obtained in the seeds of the plant are having a higher antioxidant capacity, particularly those belonging to the ethyl acetate extracts which provide with less content of phenolic compounds and flavonoids, compared to methanolic extract of the same part. The correlation analysis between the values of DPPH, TAC and FRAP indicates the viability of the three models for evaluating antioxidants from medicinal plants. Furthermore, all extracts showed good antibacterial activity against *Escherichia coli*. Our finding therefore reveals the potentials of *Calycotome villosa subsp. intermedia* as important source of natural antioxidants which may provide protection against free radicals induced damage to biomolecules.

ACKNOWLEDGMENTS

Financial support and sponsorship

This work was funded by the Sidi Mohammed Ben Abdellah University (Fez, Morocco) and the National Center for Scientific and Technical Research (CNRST-Rabat) under grant no. URAC19.

Conflict of Interests: There are no conflicts of interest.

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How to cite this article:

Elkhamlichi A, El-Hajaji H, Faraj H, Alami A, El-Bali B, Lachkar M. Phytochemical screening and evaluation of antioxidant and antibacterial activities of seeds and pods extracts of *Calycotome villosa* subsp. Intermedia. J App Pharm Sci, 2017; 7 (04): 192-198.