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Phytochemical Screening, Antioxidant and Antimicrobial Activities of *Erodium glaucophyllum* (L.) L'Hérit

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Abstract

This study deals with the phytochemical analyses, antioxidant and antimicrobial activities screening of *Erodium glaucophyllum* root bark. Organic extracts (Chloroformic Acid and Ethyl acetate) were screened for their biochemical composition as well as antioxidant and antibacterial activities. Phytochemical screening of *Erodium glaucophyllum* root bark showed the presence of flavonoids, coumarins, saponins, tannins, antracenosides, phenolics compounds and mucilage. Biochemical screening of the two organic extracts show a highest total polyphenolic content of the two solvent (78.022 ± 0.28 mg GAE/g DM in Chloroform extract and 95.468 ± 0.078 mg GAE/g methyl acetate extract). These values were recorded in the proportion of 70% MeOH. The antioxidant activity was evaluated using DPPH tests, chelation, and the total antioxidant capacity. The antibacterial activity was evaluated by the diffusion test agar vis-a-vis of six bacterial strains. The results show that the levels of polyphenolic content influenced these activities.

Keywords: *Erodium glaucophyllum*; Biochemical composition; Antioxidant; Antibacterial

Introduction

The medicinal plants appear to be rich in secondary metabolites, widely used in traditional medicine to combat and cure various ailments. Traditional medicine occupies a central place among rural communities of developing countries for the provision of health care in the absence of an efficient primary health care system. The existence of traditional medicine depends on plant species diversity and related knowledge of their use as herbal medicines [1].

Tunisia has a rich medicinal and aromatic flora (500 species out of 2163) characterized by therapeutic properties [2]. The majority of these plants occur in harsh environmental conditions (semi-arid and arid) and many are not well studied, including our target plant, *Erodium glaucophyllum* (L.) L'Hérit [3]. *Erodium glaucophyllum* is a common plant in the Nile valley, western region and deserts bordering the Mediterranean, and is known under several vernacular names Kahkul, Lesan Hamad, Kabshia, Ragma, Dahma, Murrar and Tamir and by English names Bill Stork, or Glaucus invoice stork leaves. It is used in popular medicine as oxytocic and astringent [3-5].

Some of the most important bioactive phytochemical constituents are quinones, tannins, saponins, alkaloids, flavonoids, coumarins, anthracenosides, mucilages, sterols, triterpenes and phenolic derivatives. The study of these compounds has shown their role involves in many biological activities, we can cite some examples: The coumarins are found in many plant species and have very diverse properties. They are able to prevent the peroxidation of membrane lipids and to capture hydroxyl, superoxide and peroxide radicals [6]. Tannins are molecules of high molecular weight. They are donors of protons or lipid free radicals, produced during peroxidation. This fact allows the formation of more stable tannic radicals. According to Cavin, tannins can inhibit the chain reaction of lipid auto-oxidation [7,8]. One of the primary functions of these phenolic compounds is the protection of the plant against herbivores [9]. These are phenolic compounds anti-haemorrhagic and fighting infections. Green tea is very rich in tannins, which gives it a powerful antioxidant potential [10]. In addition, hydrolysable or condensed tannins and their derivatives are the main phenolic compounds involved in the determination of astringency. Flavonoids represent a very wide range of natural compounds belonging to the family of polyphenols. Currently, flavonoids are known by remarkable pharmaco-biological activities like antiviral, antimicrobial and anticancer activities [11,12], antiallergic, anti-inflammatory, anti-thrombotic, antitumor, hepatoprotective [13] and antimutagens [14]. The importance of quinones in such basic metabolic processes as respiration: electron transfer in the respiratory chain of Mitochondria and photosynthesis has been well established [15]. Many plants used in traditional medicine worldwide contain saponins, which can often account for their therapeutic action such as insecticidal, antibiotic, fungicidal [16]. The alkaloids are known by their activities anti-inflammatory, antinociceptive and antipyretic [17]. The study of the biological power of mucilage contained in certain plants has shown that the mucilage behaves like Anti-inflammatory and antioxidative

[18]. Sterols and triterpenes inhibited colon tumor cell profiling as well as an effect on cholesterol [19-21]. The phytochemical analysis tests performed in this work were well studied previously in several works done on plants [22].

The objective of the present study is to determine the biochemical composition and the biological activity of chloroformic acid and ethyl acetate extracts of the roots of *Erodium glaucophyllum.*

Materials and Methods

Plant material

Erodium glaucophyllum was collected from the Southeast of Tunisia (Boughrara, latitude 33°86'46"N, longitude 10°25'48"E), at two periods: the vegetative stage (April) and the dormancy (October, 2014). The root bark, were separated and the plant materials were air-dried at room temperature (25℃) for 2 weeks, after which it was grinded to a uniform powder and stored in an airtight container for further use.

Preparation of extract

Different extraction conditions have been used (type and proportion of the solvent) for the preparation of the different extracts of *Erodium g.* in order to optimize and choose the most appropriate protocol. 10 g of *Erodium g.* root bark powder is homogenized in 50 ml of the hydroalcoholic mixtures at different concentrations: 100% MeOH; 70% MeOH; 50% MeOH and 30% MeOH and stirred for 24 hour, for three times successively. After filtration, the remaining residue is extracted again successively with Chloroform and Ethyl Acetate. Finally, the organic phases obtained are concentrated by rotary evaporator [23].

Preliminary phytochemical screening

Preliminary qualitative phytochemical screening was carried out with the following methods. The results of the tests were qualitatively expressed as negative (-) or positive (+).

Test for free quinones

One gram of dry material is placed in a tube with 15 to 30 mL of Petroleum Ether. After stirring and standing for 24 h, the extracts are filtered and concentrated by rotavapor. The presence of free Quinones is confirmed by the addition of a few drops of 1/10 NaOH when the aqueous phase turns yellow, red or purple color [24].

Test for tannins

0.5 g of the plant powder was boiled in 10 mL of water in a test tube and then filtered. A few drops of 0.1% Ferric Chloride was added and observed for brownish green or a blue-black coloration. This color indicated the presence of Catechic or Gallic tannins, respectively. Catechic tannins positive tests were confirmed by a red coloration appearance after adding 1 mL of concentrated Chlorhydric Acid to 2 mL of the extract and heating at 100℃ during 5 min [25].

Test for saponins

The Saponins were quantified by calculating the Froth index. To 0.5 g of extract was added 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth [26].

Test for alkaloids

200 mg of extract was diluted to 10 mL with Methanol, boiled and filtered. To 5 mL of the filtrate, 2 mL of dilute Ammonia was added. 5 mL of Chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 1 mL of Acetic Acid. This was divided into two portions. Mayer's reagent was added to one portion and Draggendorff's reagent to the other. The formation of a cream (with Mayer's reagent) or reddish brown precipitate (with Draggendorff's reagent) was regarded as positive for the presence of alkaloids [27].

Test for flavonoids

Three methods were used to test flavonoids. First, dilute Ammonia (5 mL) was added to a portion of an aqueous filtrate of the extract. Concentrated Sulphuric Acid (1 mL) was added. Yellow coloring that disappears on standing indicates the presence of Flavonoids. Second, a few drops of 1% Aluminium solution were added to a portion of the filtrate. A yellow coloration indicates the presence of Flavonoids. Third, a portion of the extract was heated with 1 mL of Ethyl Acetate over a steam bath for 3 min. The mixture was filtered and 4 mL of the filtrate was shaken with 1 mL of dilute Ammonia solution. A yellow coloration indicates the presence of Flavonoids [28].

Test for coumarins

Coumarins were tested in powdered leaves and in the Dichloromethane extract, and analysed with TLC where the solid phase was silica gel F254 (Merck 0.25 mm thickness, Merck, Schönow, Germany) and the solvent system Toluene/Ethyl Acetate (93:10). The presence of coumarins was detected in the plate under UV light (366 nm) [25].

Test for anthracenosides

Oxidized forms: 0.2 g of plant powder is macerated for 15 min in the presence of 5 mL of Chloroform. After filtration, 2 mL of 1/2 dilute Ammonia are added to the filtrate. After decanting the mixture, staining of the aqueous phase in red indicates that oxidized Anthracenosides are present [27].

Combined forms: 25 mg of plant powder is infused in the presence of 1 mL of distilled water and 1 mL of concentrated HCl. After filtration, 5 mL of the Chloroform is added and the solution is stirred. After decantation, the Chloroform phase is evaporated to dryness. To the residue was added 2 mL of diluted Ammonia. A yellow coloration, which develops and turns red by

heating in the water bath, indicates the presence of the combined forms of Anthracenosides [27].

Test for mucilages

1 mL of 10% aqueous decoctate was added to 5 mL of absolute Alcohol. After 10 min, obtaining a flocculent precipitate by mixing indicates the presence of Mucilage [29].

Test for sterols and triterpenes

For the characterization of sterols and triterpenes, the Liebermann-Buchard reaction has been adopted. The residue of the chloroform extract is dissolved in 1 mL of acetic anhydride and 1 mL of $CHCl_3$. Using a pipette, 1 to 2 mL of concentrate is placed in the bottom of the test tubes. At the contact zone of the two liquids a brownish or purple red ring is formed, the supernatant layer turning green or violet reveals the presence of Sterols and Triterpenes [30].

Test for phenolic derivatives

A few drops of $FeCl_3$ (5%) were added to the decoction extract. The appearance of green or blue color indicated the presence of the Phenolic derivatives [27].

Test for iridoids

One mL of concentrated HCl was added to the decoction extract. The occurence of black precipitate after heating the extract indicated the presence of Iridoids [31].

Total polyphenolic content (TPC)

TPC was assayed using the Folin-Ciocalteu reagent following the method based on the reduction of a Phosphowolframate-phosphomolybdate complex by Phenolic compounds to blue reaction products [32]. 0.125 mL of diluted sample extract was added to 0.5 mL of distilled water and 0.125 mL of the Folin-Ciocalteu reagent. The mixture was shaken and allowed to stand for 6 min, before addition of 1.25 mL of Na_2CO_3 solution (7%). The final volume was then adjusted with distilled water to a final volume of 3 mL and mixed thoroughly. After incubation in the dark for 90 min, the absorbance at 760 nm was read versus the prepared blank. Total Polyphenols content of fresh and dried leaves was expressed as milligram of Gallic Acid equivalents (GAE) per gram of dry basis (mg GAE/g DM.) through the calibration curve with Gallic Acid (0-500 µg/mL range). All samples were analyzed in triplicate [33].

Total flavonoid content (TFC)

TFC was measured using a colorimetric method based on the formation of the complex Aluminum-Flavonoid [32]. 0.25 mL of diluted sample extract was added to 0.075 mL of $NaNO_2$ solution (7%), and mixed for 6 min, before adding 0.15 mL of freshly prepared $AlCl_3$ solution ($6H_2O$, 10%). After 5 min, 0.5 mL of 1 mol/L NaOH solution was added. The final volume was adjusted to 2.5 mL with distilled water, thoroughly mixed and the absorbance of the mixture was determined at 510 nm. TFC was expressed as mg Quercetin equivalents (mg QE/g DM), through the calibration curve of Quercetin (0-500 µg/mL range). All samples were analyzed in triplicate [34].

Total condensed tannins (TCT)

The condensed Tannins were assayed by the Vanillin method described by Sun et al. In an acid medium, the condensed Tannins depolymerize and are transformed in the presence of Vanillin into red Anthocyanidols whose intensity is measured by spectrophotometry at 500 nm. A volume of 25 µL of each extract diluted 20 times is added to 1.5 mL of a solution of Vanillin (4%) and 750 µL of concentrated H_2SO_4. The mixture is then incubated for 15 minutes at room temperature in the dark and the absorbance is measured at 500 nm [35].

Radical-scavenging DPPH test

The free radical scavenging activity of the fruit extracts was measured by measuring the decrease in absorbance of Methanolic DPPH solution at 517 nm in the presence of the extract. The initial concentration of DPPH was 0.1 mM and the reading was taken after allowing the solution to stand for 30 min. In cases where the absorbance decreased too much (when the solution turned yellow) before the 30 min period, the sample was appropriately diluted. The antioxidant activity was expressed as:

$$\% \text{ Disappearance} = [(A_{control}-A_{sample})/A_{control}] \times 100\%$$

IC50, the amount of sample extracted into 1 mL solution necessary to decrease by 50% the initial DPPH concentration was derived from the % disappearance *vs.* concentration plot. (Concentration here means mg of fruit extracted into 1 mL solution.) The results are also expressed as ascorbic acid equivalent antioxidant capacity (AEAC) using either one of the following equations where [36]:

$$AEAC\ (mgAA/100g) = (A_{control}-A_{sample})/\ (A_{control}-A_{AA})$$

$$\times \text{conc.AA(mg/ml)} \times \text{vol extract(ml)} \times 100/\text{g sample}$$

Ferrous ion chelating

The Ferrous Ion Chelating (FIC) activity was measured by the decrease in the absorbance at 562 nm of the Iron (II)-Ferrozine complex. One milliliter 0.125 mM $FeSO_4$ and 1mL 0.3125 mM Ferrozine were mixed with 1mL sample (with different dilutions). The mixture was allowed to equilibrate for 10 min before measuring the absorbance. Sample solutions with appropriate dilutions were used as blanks as the fruit extracts may also absorb at this wavelength. The ability of the sample to chelate Ferrous ion was calculated relative to the control (consisting of iron and Ferrozine only) using the formula [34]:

$$\text{Chelating effect } \% = (A_{control}-A_{sample})/A_{control} \times 100$$

Determination of total antioxidant capacity

The total antioxidant capacities of the extracts were evaluated by the Phosphomolybdenum method as described by Prieto, et al [37]. The assay is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of a green Phosphate/Mo (V) complex at acid pH 0.3 mL of each sample

solution and Ascorbic Acid (100 mg/mL) were combined with 3 mL of reagent (0.6 M Sulphuric Acid, 28 mM Sodium Phosphate and 4 mM Ammonium Molybdate). A typical blank solution contained 3 mL of reagent solution and the appropriate volume of the same solvent used for the sample. All tubes were capped and incubated in a boiling water bath at 95°C for 90 min. After the samples had been cooled to room temperature, the absorbance of the solution of each sample was measured at 695 nm against the blank using a UV-Vis spectrophotometer. The experiment was performed in duplicates. The antioxidant activity is expressed as the number of equivalents of Ascorbic Acid [38].

Antibacterial assays

Bacterial strains: Antibacterial activity of *Erodium g.* extracts was determined against the following pathogen bacteria: *Staphylococcus aureus* (ATCC25923), *Staphylococcus epidermidis* (CIP106510), *Micrococcus luteus* (NCIMB 8166), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853). The above mentioned bacteria were cultivated and stored on nutrient broth (Bio-Rad, Paris, France).

Agar diffusion method: The bioassay used for antibacterial screening was the disc-diffusion method (Freney et al.). The bacterial suspension used to inoculate Petri dishes had a turbidity of approximately 0.5 McFarland standards.

The dry extracts were suspended in DMSO solvent at a concentration of 20 mg/mL. Then, absorbent disks (6 mm diameter) were separately impregnated with 25 mL of the different extracts, put on the surface of the inoculated plates (90 mm) and incubated at 37°C for 24 h. Standard disks of Gentamycin (10 UI) served as positive antibiotic controls. Antimicrobial activity was assessed by measuring the inhibition zone around each disk. All experiments were carried out in triplicate [37].

Statistical analysis

All analytical determinations were performed in triplicate. One-way analysis of variance was conducted using SPSS software, 17.0. A difference was considered statistically significant when $P<0.05$.

Results and Discussion

Extraction yields

Yields of the different extracts are reported in **Table 1**. Leaf extracts with the different solvents showed higher yields with Ethyl Acetate than with Chloroform.

These yields range from 7.3% to 13.3% to a maximum of 22.8% with Ethyl Acetate (70%). The Chloroform extracts have a maximum yield toward 21%. Yield values are influenced by climatic conditions essentially water deficit.

The water proportion influences yield for each extract. Extracted by 50 % chloroform gave the best yield then the other proportion (100%, 70%, 30%). For Acetate Ethyl extract the proportion 70 % present the best yield.

Table 1: Yield of extractions from Root bark of *Erodium glaucophyllum*.

Extract	Yield %	
Chloroform	100%	7
	70%	9.1
	50%	21
	30%	10.3
Acetate Ethyl	100%	13.3
	70%	22.8
	50%	7.3
	30%	8.8

Phytochemical screening

The presence of various phytochemical constituents in various solvent extracts of the roots is reported in **Table 2.** The major constituents present in the root bark of *E. gauphyllum*. The phytochemical screening carried out on the plant powder of the roots shows the presence of phenolic compounds such as flavonoids, anthocyanins, tannins and polyphenols. These roots also contain carbohydrate stores such as mucilage and O-heterosides.

Table 2: Phytochemical screening of the root bark powder of *Erodium glaucophyllum*.

Chemical constituent	Results of chemical reaction
Flavonoids (anthocyanes)	+
Coumarins	+
Saponins	+
Tannins	+
Anthracenosides combined form	+
Oxidize form	-
Phenolic derivatives	+
Free quinone	-
Mucilage	+
Iridoids	-
Sterols and triterpenes	-
Alkaloides	-
(+): Present (-): Absent	

These results show that *E. glaucophyllum* is devoid of toxic secondary metabolites and of lipid reserves such as sterols and terpenes.

The phenolic compound (flavonoid, coumarin, tannin) were the principle secondary plant metabolites determined in *E. glaucophyllum* roots. Recently, these compounds have generated considerable interest because their broad pharmacological activities. Anthocyanin helps the human immune system to work more efficiently to protect against viral infections [39]. Various studies have been demonstrated that coumarin is a potential antioxidant and its antioxidant activity is due to its ability to scavenge free radicals and to chelate metal ions [40]. Traditionally saponins have been extensively used as detergents, as pesticides and molluscicides, in addition to their industrial applications as foaming and surface active agents and also have beneficial health effects [41]. It is well known that tannins are used in healing wounds and inflamed mucous membranes [42].

TPC, TFC and TCT

Table 3: Phytochemical screening: Total Polyphenol Content (TPC), Total Flavonoid Content (TFC) and Total Condensed Tannins (TCT).

Extracts		TPC	TFC	TCT
Chloroform	100%	32.844 ± 0.634c	20.337 ± 0.042a	11.657 ± 0.054a
	70%	78.022 ± 0.28a	19.211 ± 0.086b	9.225 ± 0.059a
	50%	44.568 ± 0.31b	12.386 ± 0.062c	11.352 ± 0.035a
	30%	29.556 ± 0.18c	9.18 ± 0.032d	9.38 ± 0.054b
Acetate ethyle	100%	72.387 ± 0.102d	27.075 ± 0.025b	10.375 ± 0.015c
	70%	95.468 ± 0.078a	26.651 ± 0.023c	12.837 ± 0.011a
	50%	77.839 ± 0.12c	25.857 ± 0.064d	12.168 ± 0.053b
	30%	89.257 ± 0.016b	37.732 ± 0.032a	12.153 ± 0.013b

The *Erodium glaucophyllum* bark roots Ethyl Acetate extracts are richer in total polyphenols than the Chloroform extracts.

TPC varied significantly as a function of extraction solvent as well as the proposition in each solvent. The highest values were recorded in the concentration of 70% for the two solvent. These contents reading in Chloroform and Ethyl Acetate extracts 78.022 ± 0.28 mg GAE/g DM and 95.468 ± 0.078 mg GAE/g DM respectively. Then, the obtained results showed that TPC was dependent on the solvent nature and the equation with water. Ethyl Acetate showed higher TPC than Chloroform. Suggesting that *E. glaucophyllum* contained many polar phenolic compounds. Ethyl Acetate extraction production many contain medium hydrophobic compounds. According to Fratianni et al., the phenolic content of plants depends on different factors such as genetic, environmental and ecological conditions [42]. The **Table 3** shows that the ethyl acetate extracts contains also more flavonoid than the chloroform extracts, with a maximum content 37.732 ± 0.032 mg CE/g DM. Flavonoids play important roles in the development of plants and in the defence against pathogenic germs, predators, UV radiation and environmental stress [40]. The Chloroform extracts are poorer in tannins than the Ethyl Acetate extracts. The maximum tannin content of the Chloroform extracts and those of Ethyl Acetate are of the order of 11.657 ± 0.054and 12.837 ± 0.011 mg CE/g DM respectively.

Antioxidante activity

The antioxidant activities of the roots of *E. glaucophyllum* were assessed and confirmed by using three functional analytical methods (Radical-Scavenging Activity (DPPH), Ferrous Ion Chelating activity (FIC), Total Antioxidant capacity (TAC)). The different extracts obtained using different solvent and proportion was all-able to inhibit the DPPH radical, as well as FIC and TAC. The antioxidant potential varied widely and ranged from 5 ± 0.134 to 18.5 ± 0.531 with the DPPH method, from 10 ± 0.832 to 20 ± 0.063 with the Ferrous Ion Chelating activity, and from 14.63 ± 0.003 to 118.57 ± 0.1 with total antioxidant. A good correlation was observed between the TPC of *E. glaucophyllum* and antioxidant activities suggesting that polyphenolic compound are the major contributors to the antioxidant capacity of *E. glaucophyllum*. **Table 4** shows that the Chloroform extracts (100% and 70%) have the lowest concentration, therefore the most interesting anti-free radical activity. Concerning Ethyl Acetate extract the proportion 50% present the most important anti-free radical activity (6 ± 0.312 µg/ml).

Table 4: Antioxidante activity of two extracts of *E. glaucophyllum*: Radical-Scavenging Activity (DPPH), Ferrous Ion Chelating Activity (FIC), Total Antioxidant Capacity (TAC).

Extracts		DPPH	FIC	TAC
Chloroform	100%	5 ± 0.134c	17 ± 0.122b	118.57 ± 0.1a
	70%	5 ± 0.128c	16.87 ± 0.182b	112.762 ± 0.059b
	50%	18.5 ± 0.531a	20 ± 0.063a	107.033 ± 0.435c
	30%	6.12 ± 0.088b	15.5 ± 0.32b	96.958 ± 0.255d
Acetat ethyle	100%	12.03 ± 0.102a	17 ± 0.425a	115.825 ± 0.815b
	70%	10.5 ± 0.978b	16.5 ± 0.693a	91.959 ± 0.561c
	50%	6 ± 0.312a	10 ± 0.364b	73.649 ± 0.953d
	30%	9 ± 0.16a	10 ± 0.832b	14.63 ± 0.003a

A study by Koleva et al., suggested that the polar molecules present in plant extracts contribute to the increase of anti-radical activity [43]. According to Zimmer et al., polyphenols are efficient donors of Hydrogen to the DPPH radical, due to their ideal chemistry structure [44]. The richness of plant in phenolic compounds explains the higher concentration of inhibition of DPPH whose mechanism depends on the structural conformation of the antioxidant [45]. According to Miller et al.,

iron is essential for the transport of oxygen, respiration and enzyme activity. It is a reactive metal that catalyses oxidative damage in living tissues and cells. Ferrozine can quantitatively form complexes with Fe^{2+}. In presence of chelating agent, complex formation is disturbed by the fact that the red color of the complex is decreased [46]. Meot-Duros et al. report that the high value of antioxidant activity is closely related to the richness of phenolic compound extracts [47].

Antibacterial screening

The in vitro antibacterial effects of *E. glaucophyllum* extracts obtained with 8 extract solvent presented in **Table 5**. The results show that the diameter of the inhibition zone for the extracts reached a maximum of 14 mm for the Gram-negative bacteria and 15 mm for the Gram positive bacteria. Among the Gram-negative bacteria, *Salmonella typhimurium* appears to be the most sensitive to the chloroform extract; whose inhibition diameter varies from 10 mm to 14 mm. *Listeria monocytogenes* appears to be the most sensitive among Gram-positive bacteria; the inhibition diameter reaches 15 mm for the ethyl acetate extract of (100%). For the ethyl acetate extracts, the most important antibacterial activity is reported against *Listeria monocytogenes* by a diameter of inhibition of 15 mm, and against *Staphylococcus aureus*, *Pseudomonas aeruginosaa* and *Bacillus cereus* with an inhibition diameter of 14 mm.

Table 5: Diameters of the inhibition zones of the bacterial growth generated by the various extracts studied.

Bacterial strains	Chloroform extract				Acetate ethyl extract			
	100%	70%	50%	30%	100%	70%	50%	30%
Echerchia coli	11	9	11	9	13	7	-	8
Staphylococcus aureus	11	10	13	9	14.5	10	9	-
Pseudomonas aeruginosa	13	10	12	9	14	7	7	-
Listeria monocytogène	11	10	12.5	9	15	9	10	8
Bacillus cereus	12	11	12.5	8	14	-	8	9
Salmonella typhimurium	12	10	14	-	12.5	10.5	10	9

Despite the aridity of the environment, *E. glaucophyllum* secretes an important quantity of phenolic compounds involving its strong anti-radical activity consequently it can defend the environment stress and also survive against the association of bacteria by its antimicrobial activity. This antimicrobial activity can be ascribed to flavonoids, saponins and tannins. These phytochemical groups are known to include anticrobial compound concentration. Their presence in the plant extracts could therefore explain the observed activity in our study.

Conclusion

The phytochemical screening of *E. glaucophyllum* root bark showed the presence of many compounds such as flavonoids, coumarins, tannins, antracenosides, phenolics compounds and mucilage.

Biochemical screening of the two organic extracts show a highest total polyphenolic content values were recorded in the proportion of 70% of the two solvent (78.022 ± 0.28 mg GAE/g DM in Chloroform extract and 95.468 ± 0.078 mg GAE/g DM ethyl acetate extract). Our study clearly indicates that it is important to measure the anti-oxidant activity using various radicals and oxidation systems and to take both phenolic content and anti-oxidant activity into account while evaluating the anti-oxidant potential of plant extracts. Exploitation of these pharmacological properties involves further investigation of these active ingredients by implementation techniques of extraction, purification, separation, crystallization and identification.

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