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Extraction of pectolinarine from Linaria reflexa

Dissertation submitted to the department of chemistry for the fulfillment of Master degree in Organic chemistry

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Thanks

*All praise and thanks be to **ALLAH** , my creatore, who has facilitated my affairs and granted me so far the strenght and determination to accomplish this memorandum. **Allhamdolilaah**.*

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Dedication

To those whom ***GOD*** *honored and recommended in writing the Qur'an*

"وبالوالدين احسانا" *to* ***my Parents,*** *who were not keen on good morals and habits.*

To ***the human angel,*** *who taught me how to believe in God and trust myself and that she always supports me. She was the friend, the sister, the patron and the carer,* ***Mom*** *to the one who made me her priority even in her supplication .what lover is this who remembers you in his solitude with his lord* ***love you Mom***

To the one who held my hand when I walked for the first time, when I wrote with a pen for the first time .he was my constant help and support. To the one who never spared me anything I asked for, to the one who made my smile his strength and my tears his weakness. ***To you Dad,*** *to you* ***my father,*** *my love and my friend. collect a few words to repay the debt.*

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To the soul of my dear grandmother, ***MOULINARI Rabiaa****, who chose Algeria as a homeland, my paradied.* ***I miss you as much as I love you.***

To my dear grandfather ***GHRBI Alchik*** *, and grand marher, my beloved aunts and uncles, and all the members of my family "****GHARBI*** *family ,****BENLOKRICHI*** *family ".*

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LIST OFABBREVIATIONS

ABBREVIATIONS

- **APG** Angiosperm Phylogenetic Group
- **GBS** Global Positioning System
- **MeOH** Methanol
- **H_2O** Water
- **HCl** Hydrochloric acid
- **Na_2CO_3** Sodium Carbonate
- **$AlCl_3$** Aluminium trichloride
- **H_2SO_4** Sulfuric acid
- **CO_2H** Carboxylic acid
- **NH_3** Ammonia
- **OH** Hydroxide
- **H** Hydrogen
- **AcOH** Acetic acid
- **$CHCl_3$** Chloroform
- **$FeCl_3$** Ferric chloscide
- **RV** Rotary Evaporation
- **PPT** Total Polyphenols
- **FC** Folin-Ciocalteu
- **UV-VIS** Ultraviolet – visible
- **AG** Gallic Acid
- **Q** Quercetin

UNITS

- **°C** Degree Celsius
- **mm** Millimetre
- **mg** Milligram
- **ml** MillilitreMillilitre
- **µl** Microlitre
- **g** Gramme

- **H** Hour
- **Min** Minute
- **EAG/g** Gallic Acid Equivalent per one gram
- **EQ/g** Quercetin equivalent per one gram

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General introduction

For thousands of years, humanity has used various resources found in its environment to treat and cure most kinds of diseases [1]. The knowledge of folk healers to treat human suffering resulted from the daily practice of medicines using plants directly or indirectly as a miraculous source of healing [2]. The specific plants and methods of application used for specific diseases have been transmitted through the history of many civilizations [3].

In 2000, The World Health Organisation (WHO) estimated that about 81% of humanity use traditional herbal preparations as primary health care (World Health Organisation, 2000). Herbal medicine is still widely used and has considerable importance in international trade [4].

The African continent is endowed with immense biodiversity among the richest plants in the world, with a very high number of plants used as herbs, as natural foods and for therapeutic purposes. These plants represent a huge reservoir of potential compounds attributed to secondary metabolites which have the advantage of being of great diversity in chemical structures, and they possess a very wide range of biological activities [5].

In Algeria, few ethnobotanical surveys have been carried out in the central Sahara region, in the north-east and north-west, but similar studies in the central, north-eastern and north-western Sahara region of Algeria are almost totally absent [6].

A part of the Algerian medicinal flora remains unknown (only 146 are known) [7]. Among the known medicinal plants of the family Plantaginaceae, the genus *Linaria* is represented by

a significant number of plants used as folk remedies for their tonic, antiscorbutic, laxative, antidiabetic and diuretic properties [8], one of these species is *Linaria reflexa* Desf. (our subject) a medicinal plant known in the Mediterranean basin for the treatment of certain skin diseases . Studies on its aerial parts indicate the presence of iridoids and glycosylated flavones [9].

This study was planned to include the following chapters:

Chapter I Highlights

- The botanical taxonomy of the Plantaginaceae families according to the APG classification and the taxonomical identification of *Linaria* genus
- Traditional medicinal uses of *Linaria* species
- Biological and phytochemical studies of each *Linaria* species

Chapter II Shows

- The experimental technique that used to investigate the preliminary phytochemical composition of *Linaria reflexa* hydromethanolic extract

Chapter III Reveals

- Discussion and analysis of result and observation
- Describe the experimental and discuss the result of the biological and phytochemical study
  - The experimental study is carried out at the level of the chemistry laboratory at the University of Mohamed BOUDIAF M'sila, and the research unit of the Uninersity MENTOURI Brothres in Constantine. Biological activity at the Pasteur institute in M'sila.

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Chapter I

Literature survey

I.1. Plantaginaceae family

The Plantaginaceae are herbs or sometimes small shrubs, comprising about 250 genera and 4500 predometimes temperate species [1]. The plants of the Plantaginaceae family are usually herbaceous, sometimes shrubby or arborescent, and can be parasitic (althaea), aquatic or climbing. The leaves are alternate or opposite, the flowers are hermaphrodite, often zygomorphic, pentamerous or tetramerous. They are solitary, grouped in spikes, clusters or in panicles. The sepals are pre-flowering, valvate or imbricate. The corolla has fused petals and a valvular or imbricate pre-flowering. The stamens are usually four in number with a staminode (sometimes five or two stamens only). They are inserted on the tube of the corolla. The ovary is sterile, normally bilocular, consisting of two fused carpels. to each other. It is surmounted by a terminal style with one or two stigmas. There is often a nectariferous disc around the pistil. The ovules are numerous, unitary, anatropic to hemitropic, on axile placentas. The fruit is a dehiscent septicidal capsule, sometimes loculicidal or porricidal, rarely a berry or porricide, rarely a berry or a schizocarp. The seeds are winged or angular with a straight or slightly curved embryo in oleaginous albumen [2].

Table I.1. Classification of the Plantaginaceae family [3]

family	Tribes	Genres
Plantaginaceae	Antirrhineae	*Antirrhinum*
		Chaenorhinum
		Cymbalaria
		Kickxia
		Linaria
		Misopates
		Nuttallanthus
	Callitricheae	*Callitriche*
		Hippuris
	Cheloneae	*Chelone Collinsia*
		Nothochelone
		Penstemon
		Tonella
	Digitalideae	*Digitalis*
	Gratioleae	*Bacopa Gratiola*
	Plantagineae	*Littorella*
		Plantago
	Veroniceae	*Lagotis*
		Veronica
		Veronicastrum

I.2. presentation of the genus *Linaria*

Linaria is a genus of almost 200 species of flowering plants, one of several related groups commonly called toldfax. They are annuals and herbaceous perennials, and the largest genus in the Antirrhineae tribe of the plantain family Plantaginaceae [4].

The genus *Linaria* is distributed in the moderate climatic zones Europe, North Africa and Asia. They owe their name to their narrow leaves which resemble those of the cultivated flax. The plants of this genus are herbaceous, either erect or recumbent annuals or perennials of 10–30 cm with simple, narrow leaves (often linear and rarely lobed or toothed). Flowers solitary, axillary, in clusters or in terminal spikes. The calyx deeply divided into five unequal, often short lobes. The corolla bilabiate with a bulging tube and extended at the base by a spur.

The upper lip has two slightly reflexed lobes, the lower lip has three lobes, and the palate is prominent, closing the throat, two-lobed, brightly coloured and usually hairy. Four stamens. The fruit is an ovoid polysperm capsule [5].

Linaria species are known for their accumulation of secondary metabolites such as iridoids, flavonoids, neoclerodanes alkaloids and phenylethanoids [6]. The centre of distribution of the genus *Linaria* is the Mediterranean basin [7], in which 39 are in Algeria [8].

Figure I.1. *Different parts of Linaria plants*

I.3. Traditional medicinal uses of *Linaria*

The majority of *Linaria* species are known for their curative power. they are used in flok medicine for their various biological properties including tonic, antiseptic, laxative, antidiabetic and diuretic, as well as to treat wounds, hemorrhoids and vascular disorders [9], antispasmodic, and anti-diuretic, and infection, cholagogue and anti-colds [10]. among these species:

- ***L. vulgaris*:** it is used by the locals of Bulgaria [11], and Northeast China [12] for its laxative properties, and treatment of cystitis, haemorrhoids, rash, cough and asthma and as an expectorant [13].
- ***L. cymbalarya*:** it is a medicinal plant with diuretic, tonic and antiscorbutic properties [14], it has diuretic, tonic and antiscorbutic properties [15]. In the folk medicine of some countries, the infusion of this plant is used for its anti-haemorrhoidal, astringent and vulnerary properties. It was also used in the treatment of eczema, excoriations, chilblains, burns, scabies and ringworm [16]. Reports mention that the plant has been used successfully in India for the treatment of diabetes [17]. It was also used in the treatment of excoriations, chilblains, burns, scabies and ringworm [18].
- ***L. japonica*:** Is a perennial plant that grows in coastal areas. It is used in folk medicine as a diuretic, purgative [19] and laxative [20].
- ***L. ramosissima*:** this plant is used as flok remedy in india,in the Saurashtra region to treat urinary tract stones [21].
- ***L. buriatica*:** this plant is used in Mongolia; the flowers of this species were used as a traditional remedy for fever and edema [22].
- ***L. reflexa*:** this plant is known to be effective in treating some skin diseases [23].

❖ **Note:** Dosage is necessary and should not be given to pregnant and lactating women, as the plant may be slightly toxic [24].

Figure I.2. *Photos of some Linaria species*

I.4. Biological activities review of *Linaria* species :

The presence of certain secondary metabolites in species of the genus *Linaria* species has allowed them to have important biological properties, the most notable of which are remarkable are as follows:

- Anti-oxidant:

  Biological studies on the ethanolic extracts of *L. purperea* [25], cosmetic extract of *L. japonica* [26], chloroform, ethyl acetate and *n*-botanic of *L. tingitana* [27,28], have shown significant antioxidant properties related to the chemical composition of these species rich in polyphenols and terpenes.

- Antibacterial:

  The antibacterial activity of *L. corifolia* ethanolic extract showed that it has selective activity against Gram-positive bacteria. It also has strong effect against the acid-fast bacterium *Mycobacterium smegmatis*, evaluated in cephalosporin antibiotic

cefotoxime (CTX30) [29]. Another study carried out on the ethanolic extract of *L. scariosa* shows that it has interesting antibacterial properties [30].

- Antiviral:

  After using column chromatography to determine the chemical composition of the ethanolic extract of *L. purperea*, two compounds with extraordinary antiviral properties were isolated [25].

  Phytochemical analysis of the ethanolic extract of *L. alpina* led mainly to the isolation of some penta-cyclic triterpenes such as oleanolic acid [31], which has been identified as an anti-HIV principle [32].

- Anti-tumoral:

  Studies concerning the anticancer activity of ethanolic extracts of *L. alpina* [31] and *L. purperea* [25] reported that these two species have remarkable anti-tumour properties.

- Anti-inflammatory:

  A biological study conducted on the cosmetic extract prepared from *L. japonica* species, shows that the plant has anti-inflammatory effects [26]. Other studies on *L. vulgaris* [33], *L. purperea* [25], *L. grandiflora*, *L. genistifolia* and *L. aucheri* [34] prove that these species have a very remarkable anti-inflammatory power. Profound analyses showed that the agent responsible for the observed activities were flavonoids, in particular, pectolinarin and pectolinarigenin.

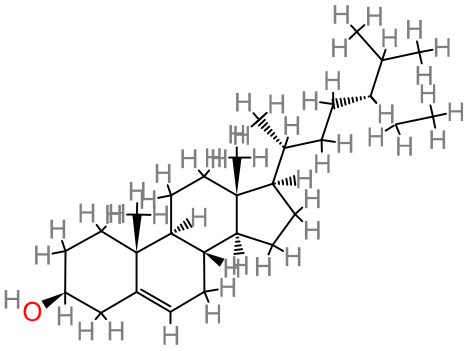
- Other activities :

  A phytochemical study carried out on the hydroalcoholic extract of *L. purpurea* showed that it has shown that the latter possesses anticoagulant, neuroprotective, hepatoprotective , anti-nociceptive and anti-diabetic activities [25].

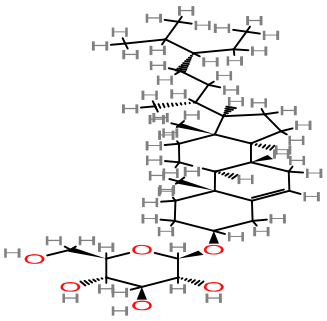
  A study on ethanolic extracts of the flowers, stems and roots of *L. Corifolia* shows that these extracts have anti-yeast effects, in particular, against *Kluyveromyces fragilis* [29]

I.5. Previous phytochemical investigation of some *Linaria* species

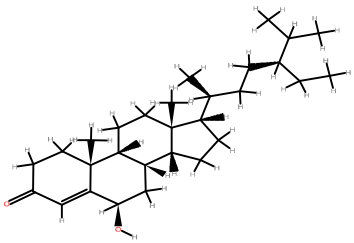
Because of the therapeutic value of some *Linaria*, more research on the phytochemistry of *Linaria* species of various secondary metabolites such as titerpenes **(1-8),** diterpenes **(9-10),** flavonoids **(11-16)** and iridoids [35-51].



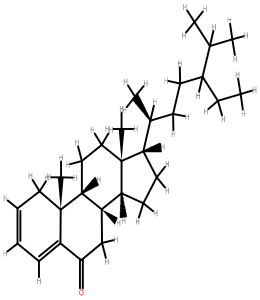
β-Sitostérol **(1)**



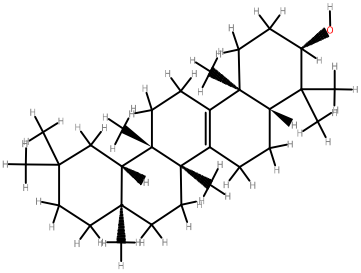
3-O-β-D-glucopyranoside (daucosterol) **(2)**



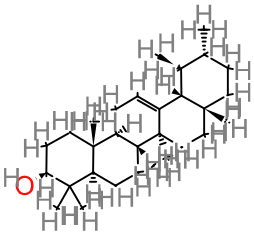
Cycloart-25-ene-3,24-diol **(3)**



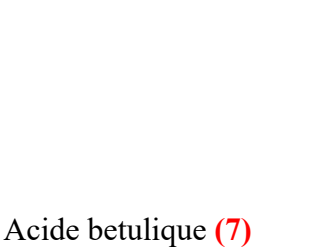
Stigmastadienone **(4)**



Isomultiflorenol **(5)**



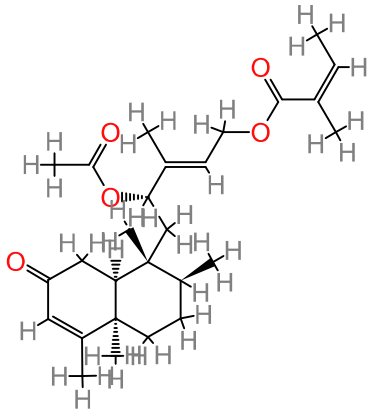
α-Amyrin **(6)**



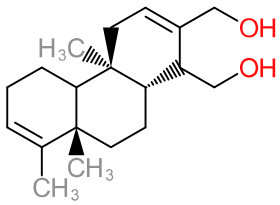
Acide betulique **(7)**

6β-Hydroxystigmast-4-en-3-one **(8)**

Figure I.3. *Titerpenes isolated from the Linaria genus*

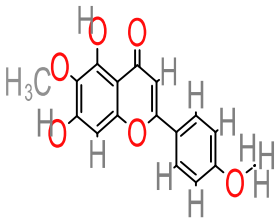


Linarienone **(9)**

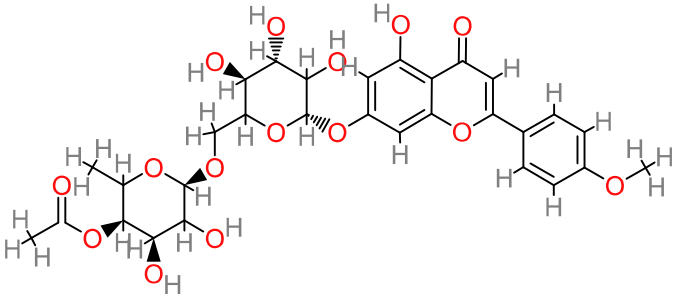


Dialdehyde linaridial **(10)**

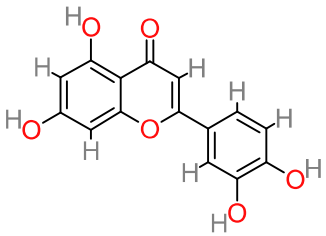
Figure I.4. *Diterpenes isolated from the Linaria genus*



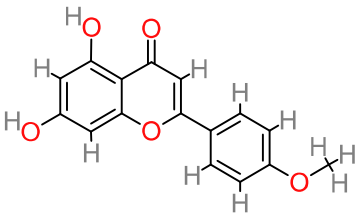
Pectolinarigenine **(11)**



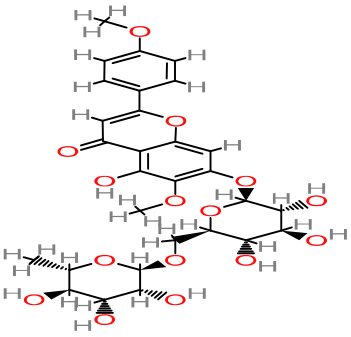
Linariine **(12)**



Luteolin **(13)**



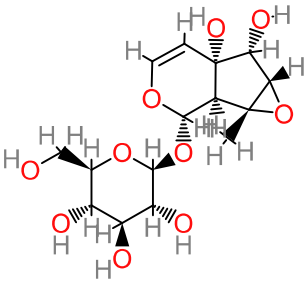
Acetine **(14)**



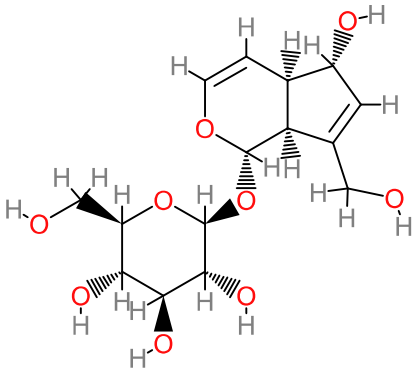
Pectolinarine **(15)**

Isolinariin B **(16)**

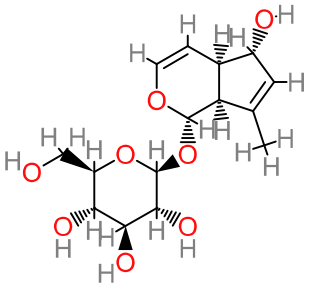
Figure I.5. *Flavonoids isolated from the Linaria genus*



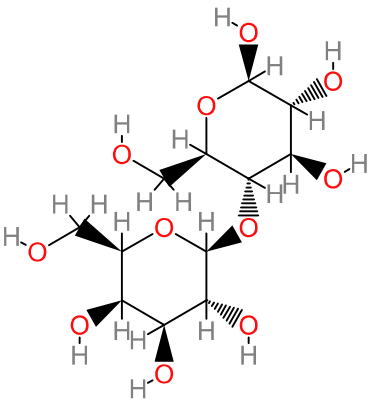
Antirrhinoside **(17)**



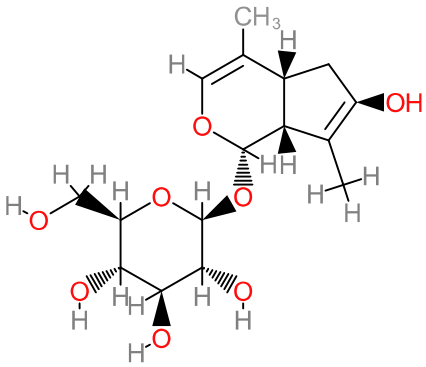
Aucubine **(18)**



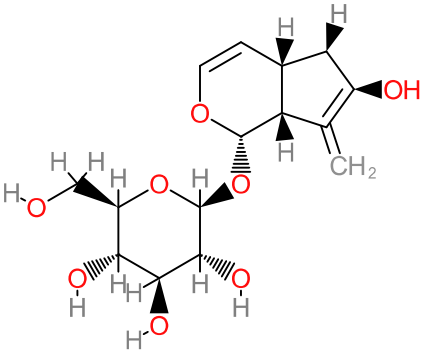
Linaride **(19)**



10-O-β-glucosylaucubine **(20)**



7-3-hydroxy-8-epi-iridodial glucoside **(21)**



Antirrhide **(22)**

6-O-senecioylantirrhinoside **(23)**

6-O-angeloylantirrhinosid **(24)**

Figure I.6. *Some iridoids isolated from the Linaria genus*

I.6. About *L. reflexa*:

I.6.1. Description of the investigated plant :

Annual plant of 10-30 cm, glabrous and gluacescent, with diffuse, flexuous procumbent stems; leaves mostly alternate, sub-sessile, acute ellipticale oval attenuated at the base, entire, flowers of a light violet with a yellowish palate closing the throat, quite large, axillary, in loose leafy clusters ;fruiting peduncles reflexed, glabrous, with lanceolate-acute lobes; corolla 15-20 mm with slender spur, acute, almoste straight,as long as it is globose capsule, shorter than the calyx,openinge at the top by valves ; honeycomb seeds [52]. (Figure I.7).

Figure I.7. . *Linaria reflexa poir*

I.6.2. Taxonomical identification of *Linaria reflexa* in plant kingdom

The botanical taxonomy of *L. reflexa* according to the APG III classification is outlined as below [53]:

Table I.2. *Botanical systematic taxonomy*

Majore taxonomique ranke	Taxon
Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopisida
Order	Lamiales
Phylum	Tracheophyta
Family	Plantaginaceae
Genus	*Linaria*
Species	*Linaria reflexa*

synonym	Oum lajrah/Chaiba

I.6.3. Why *Linaria reflexa* ?

The choice of this plant as a subject for phytochemical and biological investigations was motivated by:

- ✓ The genus *Linaria* is widespread in Algeria 39 species [8], with several species are distributed in the Algerian East.
- ✓ Its richness in secondary metabolites of the flavonoid, polyphenols and iridoid types.

I.6.4. The use in folklore medicine

A plant known as 'Oum lajrah' because of its healing power. It is also used externally in the treatment of certain dermatoses [23], It is also known as "Chaïba" [53].

I.6.5. Biological activity of *Linaria reflexa*

The antiproliferative activity of the plant results from the presence of isolinariin A, pectolinarigenin, isolinariin B, linariin, pectolinarin [45].

A biological investigation conducted on *L. reflexa* is the inhibition activity of acetylcholinesterase (AChE) of isolated flavones such as linariin, isolinariin A and isolinarin B [47].

L. reflexa can be considered as an agent in the treatment of diabetes. This anti-diabetic activity can be attributed to the presence of flavonoids, known flavonoids, known for their anti-diabetic activity and involved in the mechanism of stimulation of insulin secretion by islet β-cells and/or the inhibition of insulin degradation [48].

Good antioxidant activities have been observed for *L. reflexa* extracts, The relative activity may be related to the higher amount of flavonoids such as pectolinarinand isolinarin D [54].The in vivo test for anti-diabetic activity with aqueous (AQ) and methanolic extracts (AU) at a dose of 300 mg/kg body weight, showed strong activity (gave hypoglycaemia (-72.09%)) than that of the reference drug (300 mg/kg bw) used to treat diabetes (Glibenclamide) [54].In order to determine the anti-inflammatory and haemostatic effects of *L. reflexa*, an investigation of the anti-inflammatory and haemostatic activities was carried out. Very good results were observed especially for the hemostatic activity where a activity

where a clotting time of 45 seconds was recorded for the n-butanol extract. These effects were related to its rich chemical composition with flavones and phenolic acids [55].

I.6.6 Preliminary phytochemical screening

Plants are important source for discovering new therapeutically natural products. the present study was aimed to focus on the various phytochemical constituents from of the aerial parts of *L. reflexa*. Table I.3 shows the phytochemical screening of the aerial parts of *L. reflexa*, this analyse has revealed the high presence of alkaloids, leucoanthocyanins and flavonoids. Good presence has been detected for anthocyanins and saponins and some traces of terpenoids and unsaturated sterols, while negative results were observed for coumarins, triterpenoids and saturated sterols. This knowledge could be used for identifying the various biological potentials of this plant.

Table I.3. *Chemical composition of the petroleum ether extract of L. reflexa*

Chemical groups	Aerial parts
Alkaloids	+++
Coumarins	-
Terpenoids	±
Unsaturated sterols	±
Triterpenoids	-
Saturated sterols	-
Anthocyanins	++
Leucoanthocyanins	+++
Flavonoids	+++
Saponins	++
Tannins cathechics	+++

(+) Presence, (±) Traces, (-) Absence

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Chapter II

Experimental part

II.1. The harvest of *Linaria reflexa*

The plant material used in this study is represented mainly in the aerial parts of *L. reflexa* (stems, leaves and flowers) were collected in spring during its flowering period (March 2022) in the region of Elkhroub "Constantine" at 585 m altitude according to the following GPS coordinates: latitude 36°33'70.85"N, longitude 06°69'70.15"E. After harvesting, the plant was dried well and cut into small pieces.

Figure II.1. The aerial parts of *L. reflexa*

II.2. The used products and materials

Table II.1. *Products and materials*

Products	Materials
Deionized water (H_2O) Methanol (MeOH) Ferric chloscide ($FeCl_3$) Aqueous sodium hydroxide chloroform ($CHCl_3$) Sulphuric acid (H_2SO_4) Acetic acid (AcOH) Aqueous iron chloride Ammonia (NH_3) Folin (Na_2CO_3)	Scissors / Spatula Balance / Beecher Watch glass / Teste tube Test-tube rack Water bath (electric) Erlenmeyer / Funnel Micropipette Filter papers Rack ballon Cotton Rotary evaporator

II.3. Description of operative mode

II.3.1. Maceration

It is a conventional method which involves contacting the plant material with the solvent (with or without agitation), usually at room temperature for a specified period of time. It is based on the solubility of the compounds in the extraction solvent.

L. reflexa extraction was carried out in the laboratory of the faculty of science, M^{ed} Boudiaf University. 37.7 g of the aerial parts (dry and cut into small pieces), were macerated with a hydromethanolic solution (80%/20%) and repeated 3 times to obtain a hydromethanolic methanolic extract (Figure II.2).

Figure II.2. Maceration of the studied plant

II.3.2. Concentration of the extract

After the maceration and filtration, the obtained filtrate was evaporated to dryness under reduced pressure at 39°C using a rotary evaporator to obtain a dry extract (figure II.3).

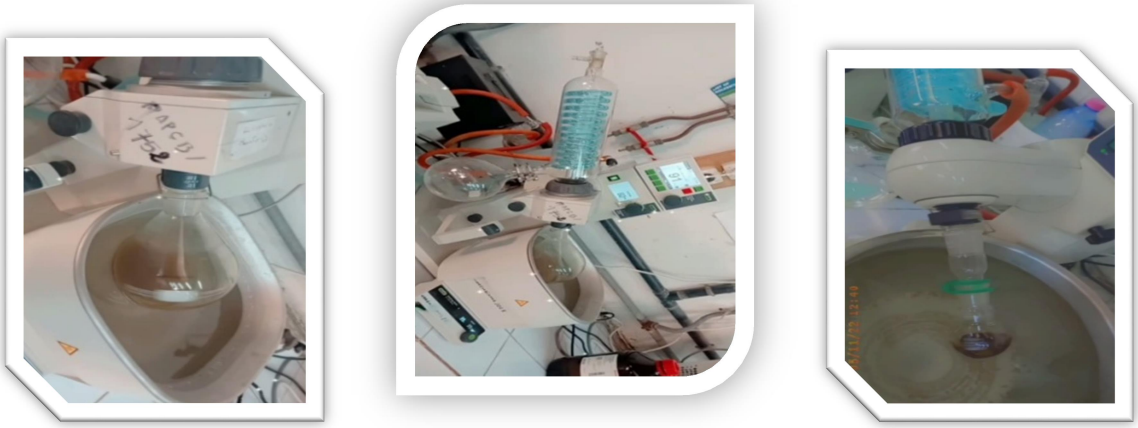

Figure II.3. *The rotary evaporation used in this study*

After processing the extract with RV device and removing H_2O and MeOH, we collected the obtained extract in a standard flask. After 24 h, we noticed the formation of a solid precipitate consisting mainly of pectolinarigenine. We filtered and separated it from the extrac.

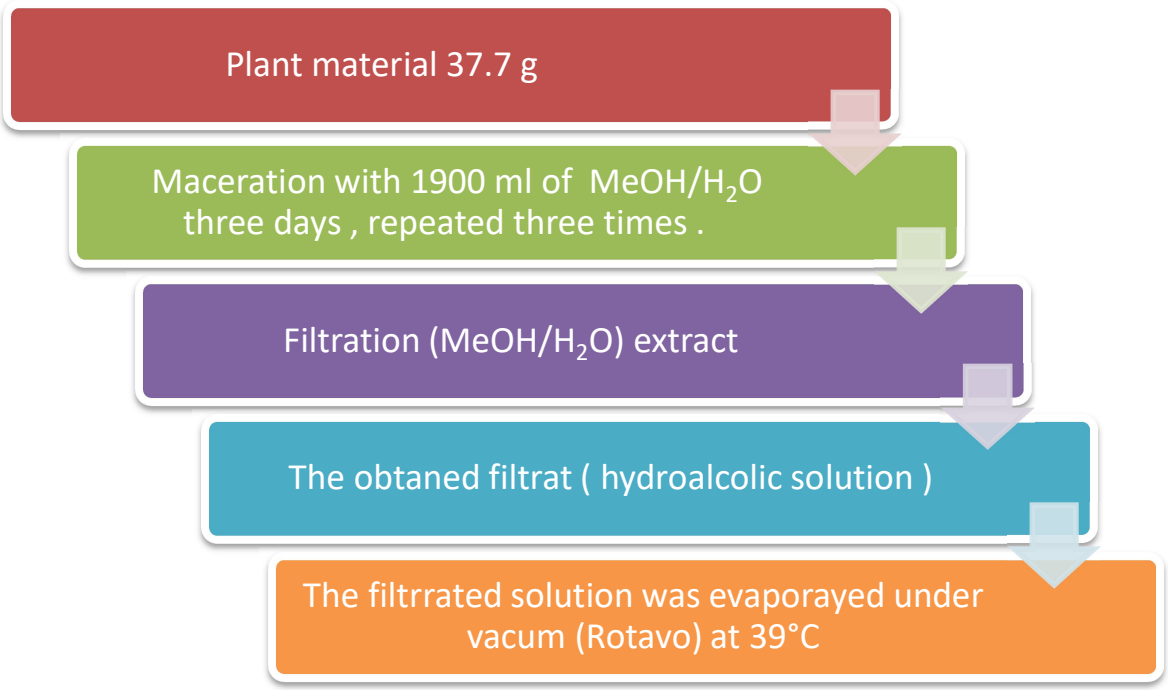

Figure II.4. *Extraction procedure of L. reflexa to obtain the hydromethanolic extract*

II.4. Determination of yield

The extraction yield was calculated by the formula given by Mahmoudi et al, 2012 which was modified:

$$R = M_{ext} / M_{ech} \times 100$$

Where R is the yield in grams, M_{ext} is the mass of the extract after evaporation of the solvents in grams, and M_{ech} is the mass of the dry plant sample in grams [1].

II.5. Analysis of *L. reflexa* extract

II.5.1. Phytochemical screening

Phytochemical screening is a qualitative test that allows the identification of the different chemical groups present in a plant material. It is based on chemical reactions that identify the presence of specific chemical substances. The classes of secondary metabolites are numerous and because of this, we have chosen only the main classes: saponins, polyphenols (tannins, flavonoids) and terpenoids (iridoids, sterols...).

The presence of the latter is attested to either by the formation of a precipitate or by the change in color of the medium. It is the appearance of the latter two that indicates the presence of the class sought [2].

- **Saponins**

  1 ml of pure extract was added to 1 ml deionized water and shaken vigorously for 30 s. Persistent frothing indicated the presence of saponins [3].

- **Water soluble phenol test**

  2 drop of 1% ferric chloride were added to 500 µl of each extract. A red color change indicated presence of water soluble phenols [3].

- **Triterpenoids**

  We used a modified version of the Salkowski test .1 ml of extract was slowly added to 400 µl chloroform, followed by careful addition of 400 µl concentrated sulphuric acid. Formation of a red/brown/purple color at the interface indicated the presence of triterpenoids [3].

- **Cardiac glucosides**

  We used a modified version of the Keller Kiliani test. 500µl of extract was added to 500 µl glacial acetic acid. A few drops of 1% aqueous iron chloride and concentrated sulphuric acid were then carefully added. The presence of a red/brown ring of the formation of a green/blue color throughout the solution indicated the presence of cardiac glycosides [3].

- **Antraquinones**

  We used a modified version of the Kumar and Ajaiycoba tests. The modified Kumar test involved addition of a few drops of concentrated sulphuric acid to 500 µl of ammonia. A rose pink color indicated the presence of free anthraquinones [3].

- **Tannins**

  We used a modified version of the ferric chloride test. Tow drops of 1 % aqueous ferric chloride reagent were added to 500 µl of crude extract. The mixture was observed for the formation of blue, blue-black, green or green-black, coloration which indicated the presence of tannins [3].

- **Flavonoids**

  We used a modified method of Kumar test. 100 µl of aqueous sodium hydroxide was added to 1 ml of each extract. The development of an intense yellow color indicated the presence of flavonoids. 100 µl of concentrated HCl was added to the solution. Return to the original color confirmed the presence of flavonoids [3].

II.5.2. Quantitative analysis and biochemical assay

Quantitative determinations of total polyphenols and flavonoids were carried out on *L. reflexa* extract. The choice of the determination of these substances was based on the fact that the majority of the biological properties of the plant are attributed to these substances classes.

II.5.2.1. Assay of total polyphenols (PPT) by colorimetry

- **a) Principe:**

The total polyphenols in the extract were determined by spectrophotometry using the Folin-Cioclateu clorimetric method. This yellow acid consists of a mixture of phosphotungstic acid ($H_3PW_{12}O_{40}$) and phosphomolybdic acid ($H_3PMo_{12}O_{40}$). The reduced Folin-Ciocalteu reagent is detectable with a spectrophotometer in the range of 690 to 710 nm .these reagent is not specific and detects all phenolic groups found in extracts including those found in the extractable proteins [4, 5].

- **b) Procedure:**

Total polyphenols (TPP) was determined, following the protocol applied by Folin-Cioclateu colorimetric : 1000 µl of aqueous plant extract (1mg/ml) were pipetted into a test tube, mixed

with 1.25 ml of FC reagent (0.2N), give quick spin .after 4-5 min, 1000 µl of sodium carbonate (Na_2CO_3) with a concentration of 75 g/l are added and the mixture was vortexed.

After incubating the reaction mixture for 2 hours at room temperature and in the dark, the absorbance is measured at 760nm by a UV-VIS spectrophotometer, all measurements being repeated twice.

- **c) Preparation of the calibration curve for Gallic acid :**

The calibration curve is performed by Gallic acid at different concentrations (1mg/ml), under the same conditions and in the same steps of the assay. The results are thus expressed as microgram of Gallic acid equivalent per one milligram dry weight (µg GAE/mg of extract).

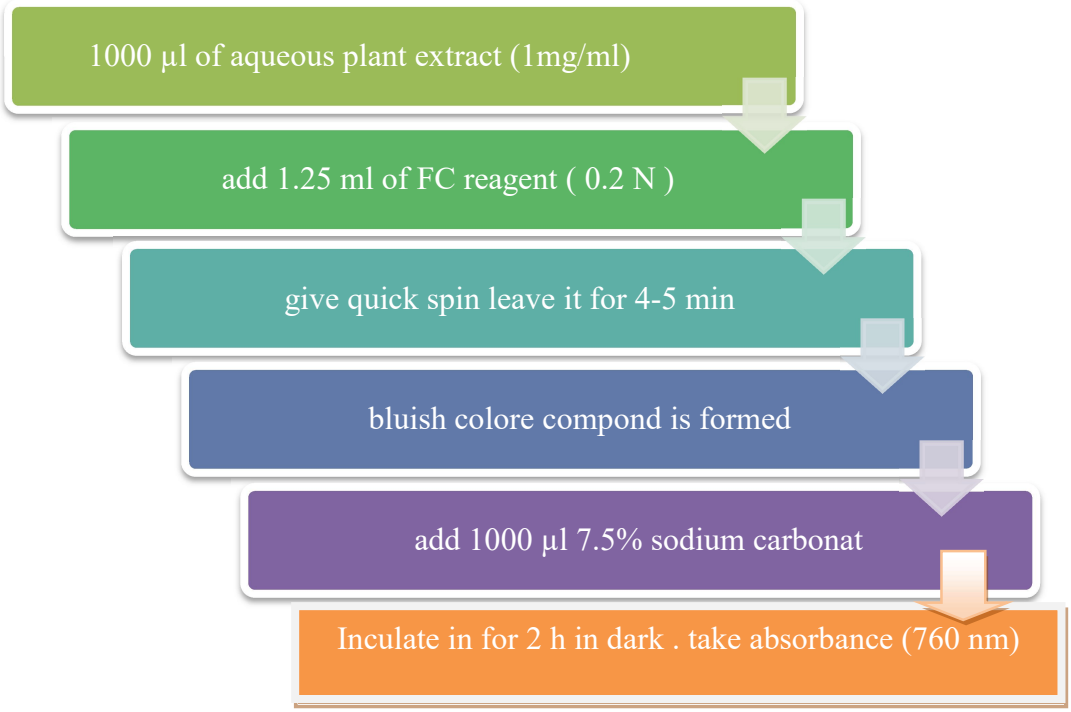

Figure II.5. *Different steps in the determination of total polyphenols (TPP)*

Figure II.6. *The blush color compound is formed*

II.5.2.2. Different steps in the determination of flavonoid

- **A / Principe :**

The determination of the flavonoid content of the methanoic extracts was carried out by the aluminium trichloride method AlCl3. Aluminium chloride forms yellowish complexes with the oxygen atoms present on carbons 4 and 5 of flavonoids [4].

- **B / Procedure**

Briefly, 1ml of the hydromethanolic extract (the extract is diluted in its original solvent), is added to 1ml of $AlCl_3$ (2% MeOH solution). After 1 h of reaction, the absorbance is read at 430nm by a UV-VIS spectrophotometer.

Flavonoid concentrations are expressed as microgram quercetin equivalent per one milligram of dry extract (µg QE/mg extract).

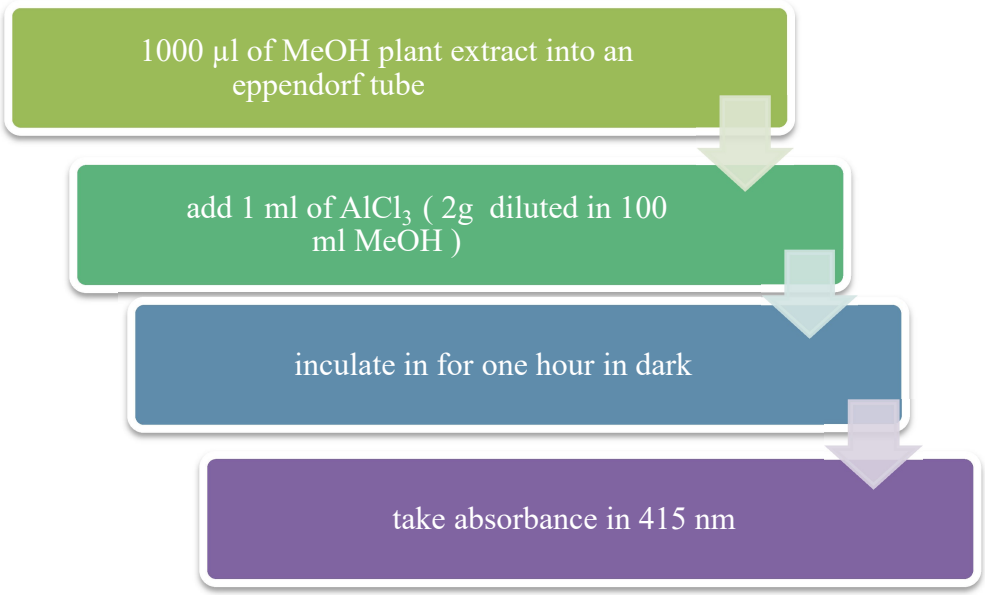

Figure II.7. *Different steps in the determination of flavonoid*

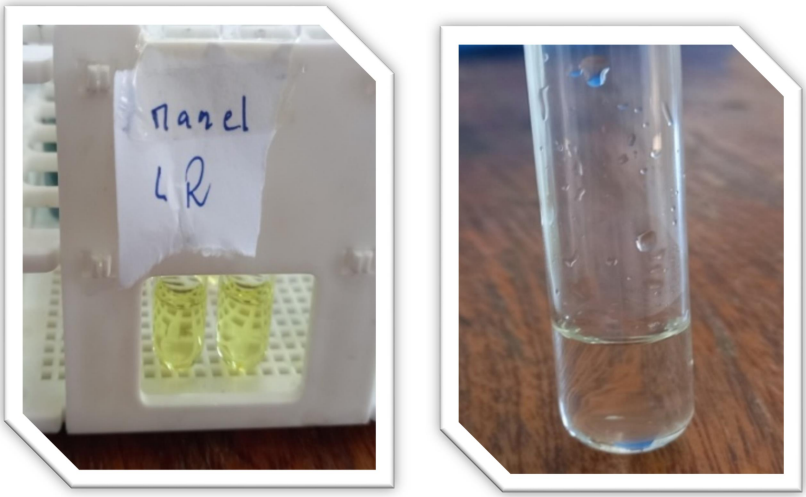

Figure II.8. *The yellowish complexe formed*

II.6. Antibacterial activity

To characterize the antibacterial activity of the extracts of the species studied, we used the agar diffusion method. This is a qualitative technique based on the measurement of the diameter of the apparent inhibition halos around the discs loaded with plant extract.

- The bacterial strains: All the strains come from the Pasteur Institute in M'sila.
  - ✓ - *Staphylococcus aureus*: ATCC 29523
  - ✓ - *Pseudomonas aeruginosa*: ATCC 27853
  - ✓ - *Escherichia coli*: ATCC 29522

Bacterial strains are maintained by plating on nutrient agar (MHA) by promoting their growth for 24 h in the dark at 37°.

After incubation, 4-5 well-isolated colonies are transferred with a platinum loop to a tube containing 9 ml of sterile physiological water is placed on the surface of petri dishes of petri dishes containing Agar. A spread of this volume is made with a sterile swab, rotating the box at approximately 60° after each application in order to purpose of having an even distribution of the inoculum. WATMAN paper discs (6mm) impregnated with a defined quantity 200 µl of each extract are placed on the surface of the agar medium previously inoculated with the bacteria. . The boxes are then incubated at 4°C for 4 h to allow good diffusion of the supernatant. Then the incubation is done at 37°C for 24 h. The antibacterial activity is revealed by the appearance of the inhibition zones around the discs. The reading: Any antibacterial activity would be manifested by the formation of an inhibition diameter which is then measured in mm with a ruler.

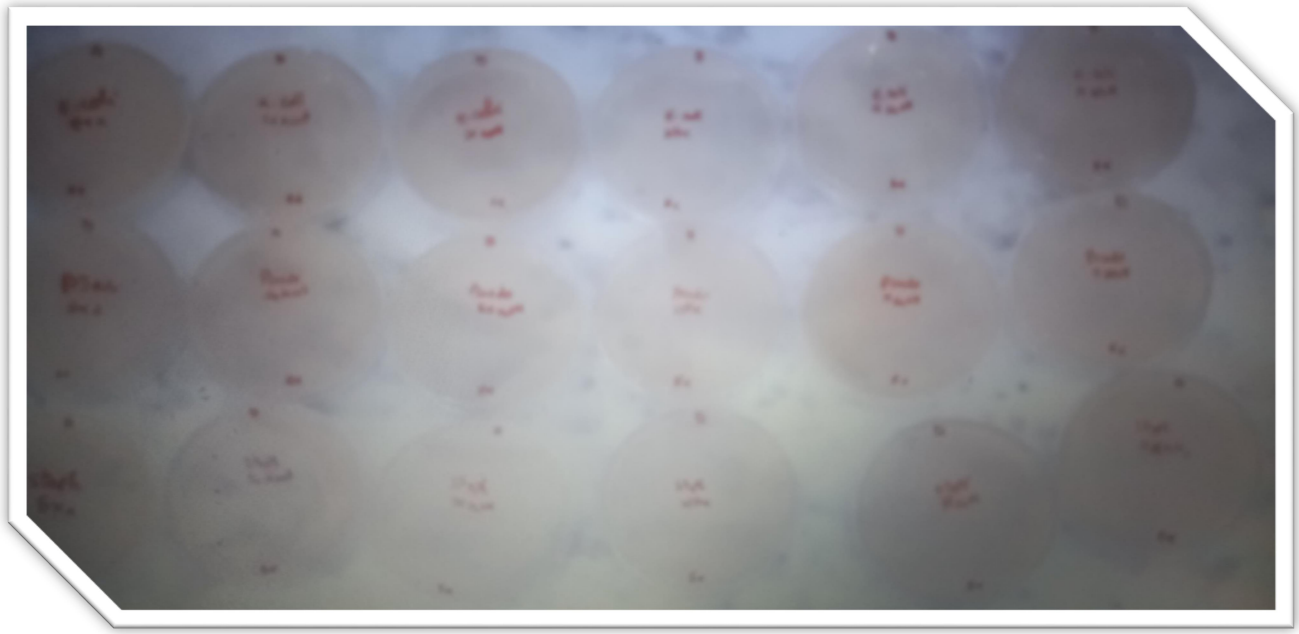

Figure II.9. *Petri dishes of the three bacterial strains used after incubation*

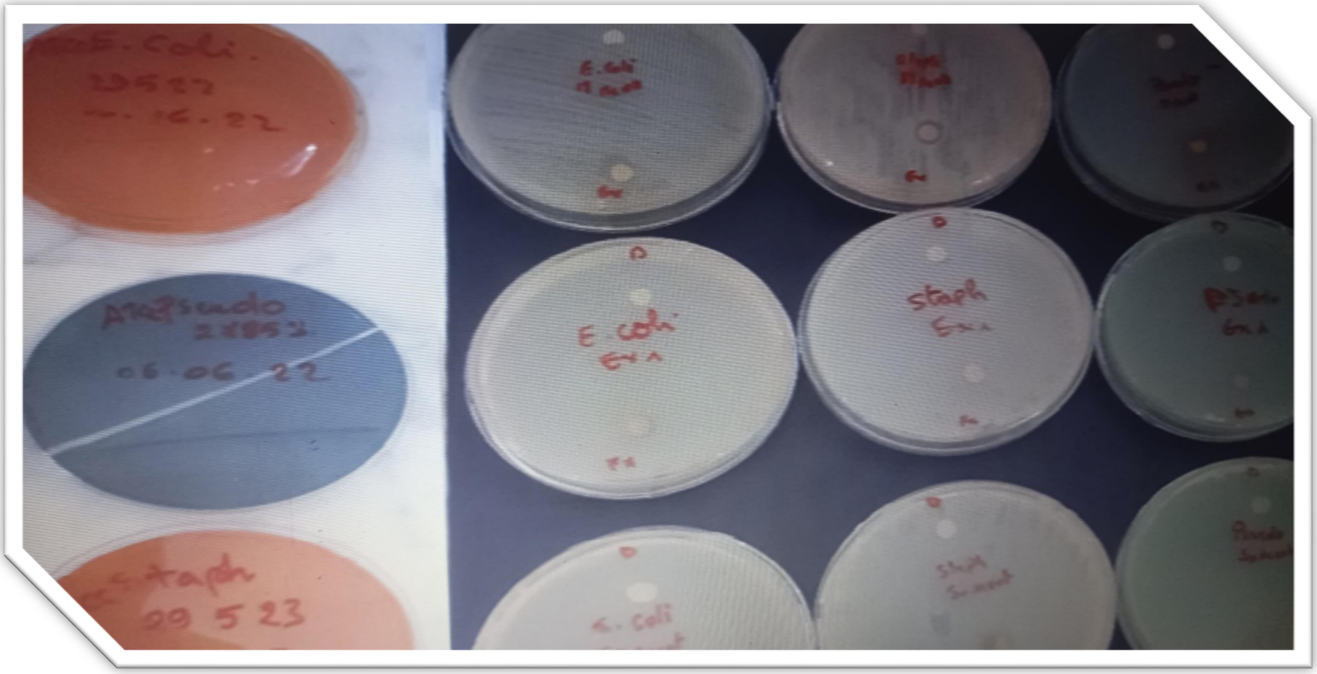

Figure II.10. *Preparation of the extracts inoculum*

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Chapter III

Results and discussions

III.1. Determination of yield

The yield was calculated as below

$$R = \frac{Mext}{Mech} \times 100 = \frac{3.82}{37.7} \times 100 = 10.13\%$$

R ≡ 10 %

This is a good yield if we compared to other plants from the genus *Linaria* [1].

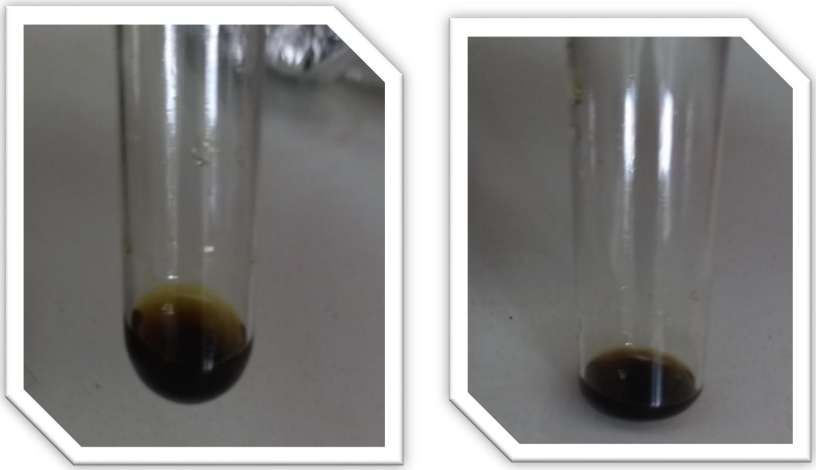
III.2. Analysis of *L. reflexa* extract

III.2.1. Phytochemical screening results

The phytochemical screening of the studied plant allowed the identification of the different classes of secondary metabolites, the results of which are:

- **Saponins :**

Observation: the presence of foam (frothing) indicates the presence of saponins.



a. After **b. Before**

Figure III.1. *Screening of saponins in L. reflexa's hydromethanolic extract*

According to figure III.1, we noticed the absence of the frothing (foam), and this indicated the absence of saponins in L. reflexa's extract.

- **Water soluble phenol test:**

Observation: a change in the extract color to red indicates the presence of water soluble phenols.

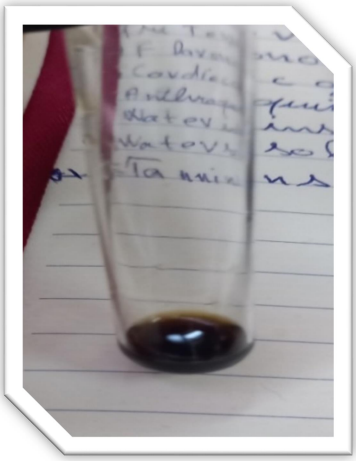


Figure III.2. *Screening of water soluble phenol in L. reflexa's hydromethanolic extract*

After the figure III.2, we noticed that the color has not changed (absence of a red color), which indicated the absence of water soluble phenols in *L. reflexa's* extract.

- **Triterpenoids :**

Observation: the formation of a red/brown/purple color at the interface indicated the presence of triterpenoids.

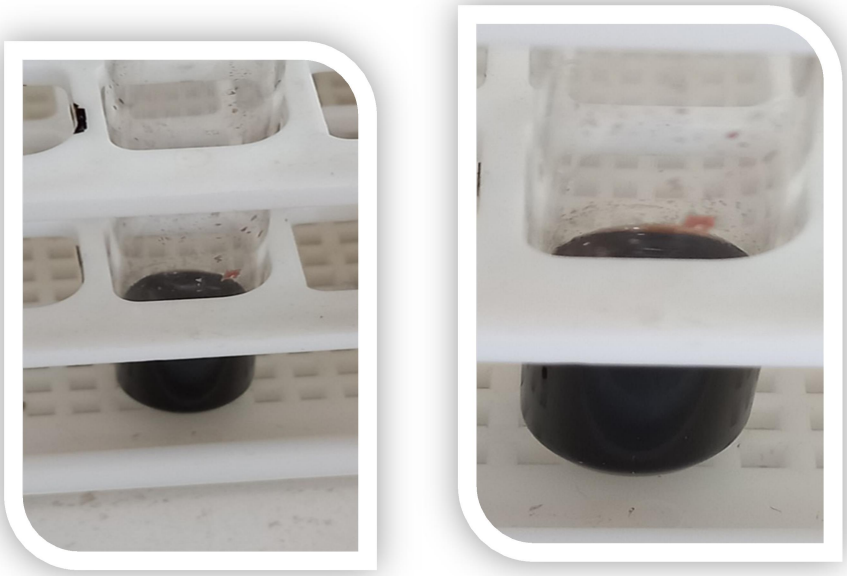


Figure III.3. *Screening of triterpenoids in L. reflexa extract*

We noticed a change in the extract color to red, and this indicates the presence of triterpenoids in *L. reflexa* extract.

- **Cardiac glycosides:**

Observation: the presence of a red/brown ring of the interface indicated the presence of cardiac glycosides.

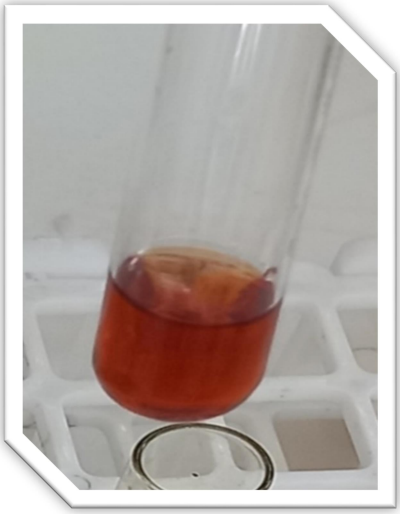


Figure III.4. *Screening of cardiac glycosides in L. reflexa extract*

According to figure III.4, the observation of a red coloration and the presence of a brawn ring indicate that the hydromethanolic extract of *L. reflexa* contains a good amount of cardiac glycosides.

- **Antraquinones:**

Observation: a change in the color to rose pink indicates the presence of free antraquinones.

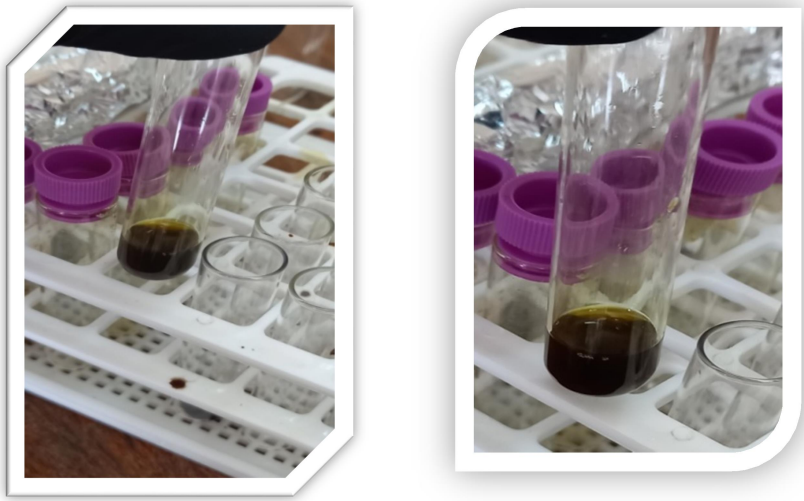


Figure III.5. *Screening of antraquinones in L. reflexa extract*

From Figure III.5, the absence of color alteration is clear, indicating the absence of the desired class in *L. reflexa* extract.

- **Tannins :**

Observation: The formation of green coloration which indicated presence of tannins.

Figure III.6. *Screening of tannins in L. reflexa extract*

From the experiment performed in a test tube (Figure III.6), we observed the change of color to green which indicates the presence of tannins in the extract of *L .reflexa*.

- **Flavonoids :**

Observation: The development of an intense yellow color indicates the presence of flavonoids.

Figure III.7. *Screening of flavonoids in L. reflexa extract*

According to the color change observed in figure III.7, it can be said that *L. reflexa* extract contains a good amount of flavonoids.

All the results are presented in the next table.

Table III.1. *Result of phytochemical screening of L. reflexa extract*

Sought-after class	Result
Saponins	-
Water solible phenol test	-
Triterpenoids	++
Cardiac glucosides	+++
Anthraquinones	++
Tannins	-
Flavonoids	++++

(-): negative; (+): slightly positive; (++): positive; (+++): strongly positive

Table III.1. show the tested phytochemical constituents for *L. reflexa* extract and revealed the high presence of tannins, flavonoids and cardiac glycosides, while tests showed the absence of

antraquinones and water solible phenols. These results are in accordance with the literature of the genus *Linaria* of which terpenoids are the most frequented molecules **[1, 2]**.

III.2.2. Quantitative analysis and biochemical assay

A dosage of total polyphenols and flavonoids was carried out in order to characterize the hydromethanolic extract prepared from dry aerial parts of *L. reflexa*. The total polyphenols content was determined by the Folin ciocateu method, where Gallic acid was used as the standard (760 nm). For flavonoids, the assay was carried out using the aluminum trichloride method.

Results are represented in the table III.2, the diagrams, and the calibration ranges in the figures (III.8; III.9).

Table III.2. *The content of phenolic and flavonoids*

Extract	Flavonoids [a]	Polyphenols [b]
Extract+ H_2O	/	4.60
Extract + MeOH	19.76	/

[a] µg equivalent of Gallic acid per milligram of extract.
[b] µg equivalent of Quercitrine per milligram of extract.
Values represent the mean of 2 measurements ± SD.

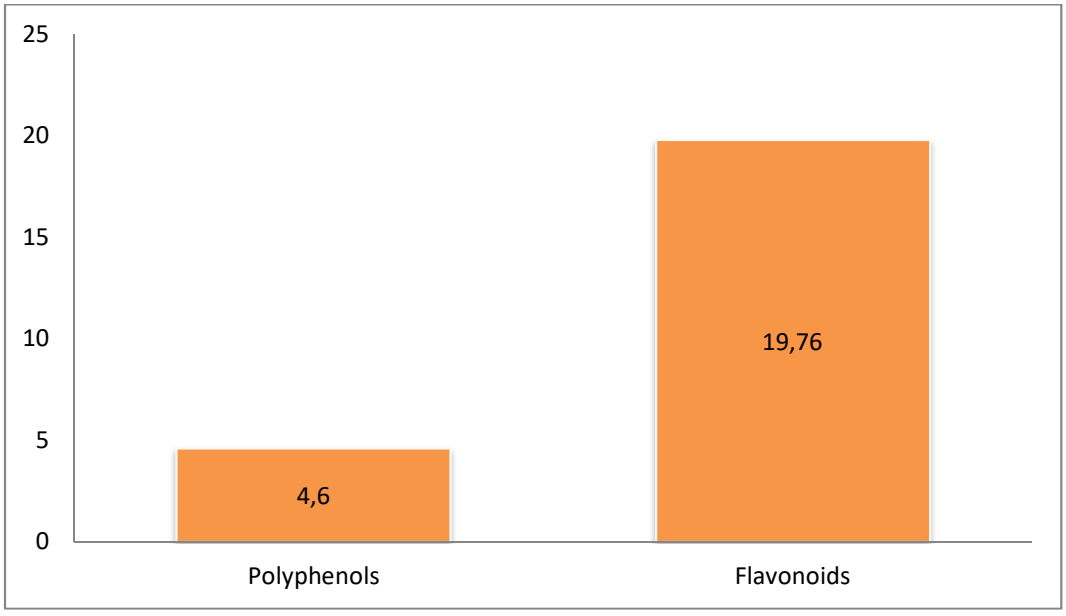


Figure III.8. *The content of polyphenols and flavonoids (µg/Eq Standard /mg) in the extract*

The results obtained showed the presence of total polyphenols and flavonoids in the studied extracts, and based on these results, a discrepancy in the content was observed. Where we find that the amount of polyphenols (4.6 µg EAG/ mg EE) is low compared to flavonoids (19.76 ug QE/ mg EE).

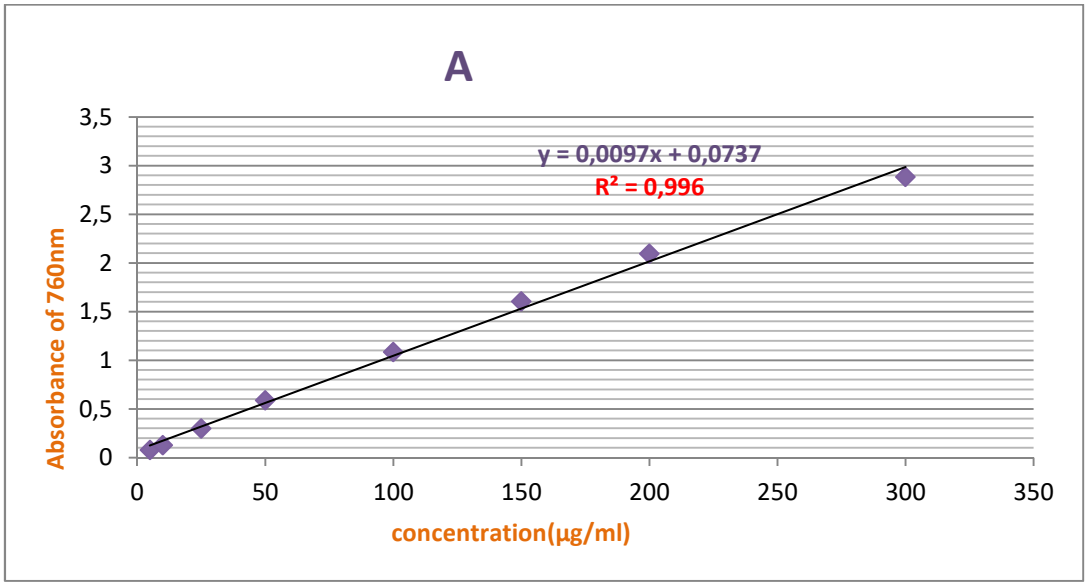


Figure III.9. *Gallic acid sealing line (mean ± SD of three tries)*

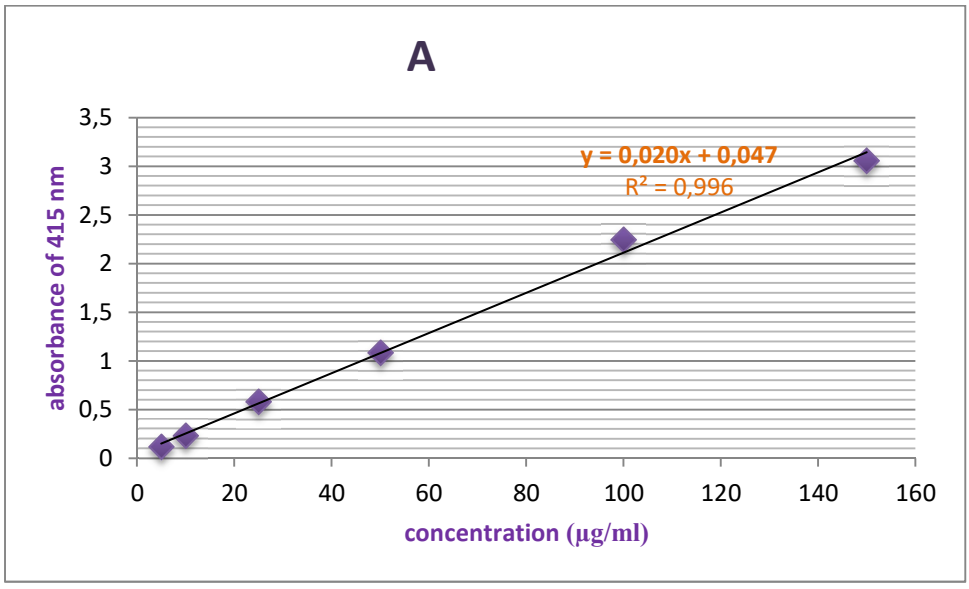


Figure III.10. *Quercitrine sealing line (mean ± three trials)*

III.3. Biological activity

III.3.1. Antibacterial activity

Antibacterial activity is determined by measuring the diameter of the inhibition zone produced around the discs after 24 h incubation at 37°C. The obtained results are shown in the following table:

Table III.3. *Diameter of the inhibition zone (mm) of L. reflexa extract*

Strain	LR extract
E. Coli	0
P. aeruginosa	0
S. aureus	0

We studied the antibacterial activity of the extracts against several reference strains.

The tests we performed show that the strains :

- ✓ - *Staphylococcus aureus*: ATCC 29523
- ✓ - *Pseudomonas aeruginosa*: ATCC 27853
- ✓ - *Escherichia coli*: ATCC 29522

The antibacterial activity showed that no effect was observed for the extract against the three strains.

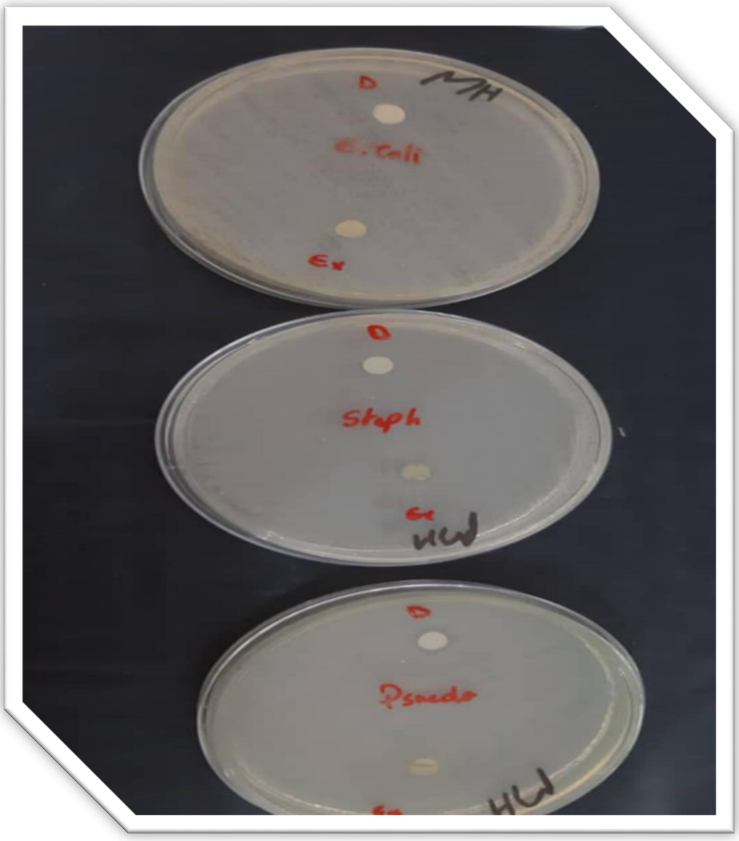


Figure III.11. *Antibacterial activity of L. reflexa extract*

References

[1] Cheriet T, Mancini I, Seghiri R, Benayache F, Benayache S. Chemical constituents and biological activities of the genus *Linaria* (Scrophulariaceae). Nat Prod Res. 2015, 29:158–1613

[2] Tundis, R., et al., Potential antitumor agents: Flavones and their derivatives from *Linaria reflexa* Desf. Bioorganic & Medicinal Chemistry Letters, 2005. 15(21): p. 4757-4760

General Conclusion

Algeria has an exceptional floristic diversity, several species are used in traditional pharmacopoeia as a remedy for various diseases and infections.

The aerial parts of *L. reflexa,* which is a North African folk medicine herb, was the subject of this study focusing on the phytochemical and antibacterial screening looking for chemical composition and its relationship with the biological effect. The phytochemical study was carried on two parts, the first one was about the qualitative analysis that were carried out and revealed the presence of flavonoids, triterpenoids, cardiac glycosides and anthraquinones with the absence of tannins, water soluble phenols and saponins in the hydromethanolique extract. The second part was about the quantitative analysis, evaluating the contents of flavonoids and polyphenols. Remarkable results were noticed especially for flavonoids (19.76 ug QE/ mg EE). This result confirms what we know about the presence of many flavonoids and in particular glycosylated flavones in the genus *Linaria*. This genus is known for the presence of some typical flavones, in particular, pectolinarigenin, the one we found it as yellow precipitate.

The biological investigation was about the antibacterial effect of *L. reflexa* hydromethanolic extract against three bacterial strains named *E. coli*, *P. aeruginosa* and *S. aureus*. Negative effect was observed for the extract against all the tested strains. This result is little bit know for the genus *Linaria* where very few studies published a good antibacterial effect for some *Linaria* species.

Abstract

Natural extracts derived from plants contain a variety of biologically active molecules. In this context we have tried to evaluate the antibacterial activity of the extract prepared from *Linaria reflexa*, and to study its phytochemical properties.

This extract was obtained by maceration in a polar solvent (MeOH/H_2O 8/2) giving a value of 10.13 %.

The qualitative analysis of this extract by preliminary phytochemical tests revealed the presence of several compounds including phenols and flavonoids, this was confirmed by the quantitative analysis, which was mainly based on the determination of the concentration of phenolic compounds (4.6 µg EAG/ mg EE) and flavonoids (19.76 ug QE/ mg EE) for the studied extract.

The antibacterial activity showed that no effect was observed for the extract against the three strains.

Résumé

Les extraits naturels issus des végétaux contiennent une variété de molécules biologiquement actives. Dans ce contexte nous avons tenté d'évaluer l'activité antibactérienne de l'extrait préparé à partir de *Linaria reflexa* et d'étudier ses propriétés phytochimiques.

Cet extrait a été obtenu par macération dans un solvant polaire (MeOH/H_2O 8/2) donnant une valeur de 10.13 % .

L'analyse qualitative de cet extrait par des tests phytochimiques préliminaires a révélé la présence de plusieurs composés, dont des phénols et des flavonoïdes, cela été confirmé par l'analyse quantitative, qui repose principalement sur la détermination des concentrations de composé phénoliques (4.6 µg EAG/ mg EE) et flavoniques (19.76 ug QE/ mg EE) pour l'extrait étudié.

L'activité antibactérienne a montré qu'aucun effet n'a été observé pour l'extrait contre les trois souches.

الملخص

تحتوي المستخلصات الطبيعية المشتقة من النباتات الطبية على مجموعة من الجزيئات النشطة بيولوجيا.

في هذا السياق حاولنا تقييم النشاط المضاد للبكتيريا للمستخلص المحضر من نبات الليناريا ريفلكسا و دراسة خصائصه الكيميائية النباتية.

تم الحصول على هذا المستخلص بالنقع في مذيب قطبي (ميثانول/ماء) عائده بقيمة % 10.13 .

أظهر التحليل النوعي لهذا المستخلص بواسطة الإختبارات الكيميائية الأولية وجود عديد المركبات منبما في ذلك الفينولات و الفلافونويد. وهذا ما أكده التحليل الكمي الذي يستند أساسا إلى تحديد تراكيز المركبات الفينولية (4.6 ميكروغرام مكافئ الغاليك /ملغ) و الفلافونويدات (19.76 ميكروغرام مكافئ الكارسين /ملغ) للمستخلص المدروس .

كشفت النشاط المضاد للبكتيريا أنه لم يلاحظ أي تأثير للمستخلص ضد السلالات الثلاثة المستخدمة في هذه الدراسة.