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PHYTOCHEMICAL SCREENING AND EVALUATION OF THE ANTIBACTERIAL ACTIVITY OF *GALACTITES TOMENTOSA* EXTRACT

Boughrara Boudjema[1*], Bouarioua Azeddine[2], Bouabibsa Ikram[3] and Didaoui Linda[3]
[1]Materials Physico-chemistry Laboratory, Science and Technology Faculty, Chadli Bendjedid El Tarf University, P.B. 73, El Tarf 36000, Algeria
[2]Department of process engineering, university Annaba, Algeria
[3]Department of environmental engineering, National polytechnic School, Algeria
* E-Mail:b.boughrara@univ-eltarf.dz
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ABSTRACT

The aim of current study was to investigate a wild plant, known by purple milk thistle (*Galactites tomentosa*), very abundant in El-Kala National Park and the Mediterranean basin.
This study was designed to evaluate the antibacterial activity effects of the *Galactites tomentosa.* The extract was further analyzed by spectrophotometry, to measure the sugars (5,51 ± 0,04 mg/g DM), the proteins (14,86 ± 0,30 g/100g DM), polyphenols (12,24 ± 0,10 GAE mg/g DM), and chlorophyll pigments (1,13 ± 0,20 mg/g FM). This work is considered as an introduction to the valuation of El-Kala National Park (EKNP) plants, for the development of the food and pharmaceutical industry.
***Keywords* :** *Galactites tomentosa* Asteracae, sugars, proteins, polyphenols, bioactive substances.

Introduction

For centuries and even millennia, man drew from his environment the knowledge necessary for his survival and well-being. Even before he had the use of language, he could transmit the experience of medicine and all types of natural remedies to finally create a link of complementarity with nature and develop what we now call traditional medicine (Van Wyk & Prinsloo, 2020). As a result, humans have often had recourse to traditional medicine which generally has less toxicity, fewer contraindications and little risk of overdose, it based on the use of plants and their active substances (Li *et al.*, 2020). In turn, the animal has not waited for humans to eat their fill, it finds in nature what suits them: grass, acorns, wild plants, but with breeding the situation has changed. And it is the breeder who manages the specific feed of the animals, which must contain daily supplies of energy, proteins, vitamins, minerals and vegetable fiber, there is more and more talk of their interest in animal health. Farmed plants work by stimulating the adaptive capacities of animals to environmental factors. So, if they are used correctly and at the right times, make it possible to have reactive animals at the right time and therefore less sensitive, consequently they potentially declare fewer pathologies, we cantherefore hope to declare less economic losses and less treatment in particular antibiotics, which remains a priority in animal health(Alba *et al.*, 2020). Plants contain hundreds or even thousands of active chemicals. Often determining the action of a plant in detail is very difficult (Medeiros-Neves *et al.*, 2018). The plants used for the development of new active ingredients must be the subject of rigorous scientific investigative work which requires multidisciplinary teamwork (chemists, biologists, pharmacists, veterinarian, etc.) (Fadel *et al.*, 2020). Indeed, next to the classic primary metabolites (carbohydrates, proteins, lipids, and nucleic acids), they also synthesize a large number of other metabolites indirectly essential for plant life, called "secondary metabolites". The search for bioactive molecules from plants can be carried out according to several strategies: an ethno-pharmacological approach which consists in using the knowledge of traditional medicines, a chemo-taxonomic approach which is interested in taxa known to contain secondary metabolites particular species, or a systematic screening of species, or any combination of the preceding (Armah *et al.*, 2018). According to the World Health Organization (WHO), nearly 4,576 plant species are used in Africa, of which more than 1,500 genera representing 192 families are used in traditional African medicine (Van Wyk, 2020).

Algeria is characterized by its floral diversity: Mediterranean, Saharan and Paleo-Tropical flora, estimated at more than three thousand species belonging to several botanical families. These species are mostly spontaneous with a significant number (15%) of endemic species. In this study, it was for us to contribute to a better knowledge of the plant of the Asteraceae family (*Galactites tomentosa*), which grows in the North East region of Algeria (El-Tarf) in the wild, a plant that can be used by breeders for feeding cattle with the aim of good milk production and fattening them, to measure the bioactive molecules and to evaluate its biological activities and to assert the nutritional power of this plant.

Material and Methods

Plant material and harvest site

The selected species *Galactites tomentosa,* was collected in the region of Rmal souk, about 30 km east of the

wilaya of El-Tarf which is part of the El-Kala National Park (EKNP) Algeria.

Botanical identification

The species was identified by the Forest Inspector, Head of Conservation (EKNP) W. El-Tarf.

Botanical description

Galactites tomentosa is very thorny and very variable in height from 20 to 80 cm, its stem is much branched at the top, tomentose. The leaves are long, narrow, deeply toothed, almost winged, thorny, cottony below and green above, but laden with milky spots. The flower heads of G. tomentosa are quite large (about 3cm in diameter), with an involucre formed by numerous erect bracts, terminated in long thorns, often surrounded by a spider veil. All the flowers are tubular. The exteriors are large and radiant, purple or purplish in color (there are also specimens with almost white flowers), sterile, deeply cut into five rigid strips while the interiors are smaller. The fruits are brown and glabrous achenes, roughly cylindrical, with feathery bristles.

Table 1: Botanical description of *Galactites tomentosa*

Reign	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Asterales
Family	Asteracae
Genus	*Galactites*
Species	*tomentosa*

Use of *Galactites tomentosa*

Galactites tomentosa is well known in agriculture for its composition rich in proteins and milky juice and therefore this plant is the main food for lactating animals.

Preparation of samples

Drying: The *Galactites tomentosa* plant is saturated with water, it is difficult to do natural drying and is replaced by artificial drying. After harvesting the plant and cleaning it, it is placed in the oven for 48 hours at 50 °C.

Conservation

After drying, the best way to store dried plants is to place them in a paper bag, store them away from light and moisture. Under these storage conditions, plants can retain all of their properties until the date of extraction or further testing.

Phytochemical screening

Sapnosides (foam test)

Following the protocol (Chatoui *et al*., 2016).

- We put 1 g of the dry powder in a flask in which 10 ml of distilled water are added.
- We put in a water bath for 5 minutes.
- After filtration, the mixture is filtered, 2.5 ml of the filtrate is added to 10 ml of distilled water in a test tube.
- The tube is shaken vigorously for 30 s then left to stand for half an hour.
- A cellular foam reveals the presence of saponins.

Flavonoids

Following the protocol(Chatoui *et al*., 2016).

- 10 g of the powder are macerated in 150 ml of 1% HCl for 24 hours.
- After having filtered the mixture, the following test is carried out.
- Take 10 ml of the filtrate, make it basic by adding NH_4OH.
- After three hours, the appearance of a light-yellow color at the top of the test tube indicates the presence of flavonoids.

Tannins

Following the protocol (Chatoui *et al*., 2016).

- 1 g of dry powder is placed in 20 ml of 80% NaOH.
- After 15 minutes of stirring, the extract is filtered and placed in a test tube.
- Adding drops of a 1% $FeCl_3$ solution makes it possible to detect the presence or absence of tannins.

Alkaloids

Following the protocol(Rg *et al*., 2018).

- 1g of the powder of the dried and ground plant is mixed with 10 ml of 5% HCl in a container.
- After half an hour of maceration. The mixture is filtered and a few drop of Mayer's reagent is added to the filtrate.
- The appearance of a yellowish-white precipitate indicates the presence of alkaloids.

Volatile oils:

Following the protocol(Rg *et al*., 2018).

- 1 g of the powder is macerated in 40 ml of distilled water with constant stirring for 30 minutes.
- After filtration of the extract, 2 ml of the filtrate are shaken with 0.1 ml of dilute NaOH and a small amount of dilute HCl. The formation of a white precipitate indicates the presence of volatile oil.

Quinones

Following the protocol (Rg *et al*., 2018).

- 1 g of the ground powder is placed in a tube with 15 to 30 ml of petroleum ether.
- After stirring and standing for 24 hours, the extract is filtered and then concentrated with a rotary steamer.
- Add 2 ml of NaOH diluted in 1 ml of plant extract. The blue-green or red color formation indicates the presence of quinones.

Dosages by Spectrophotometer

All dosages are made by jenway 3600 spectrophotometer.

Dosage of Soluble Sugars

Take 100 mg of plant powder with 3 ml of 80% ethanol. The whole is left at room temperature for 48 h, then the ethanol is evaporated off, then 20 ml of distilled water is added to the dry residue. 4 ml of anthrone reagent is placed in a test tube containing 2 ml of the extract obtained, then it is placed in a water bath at 62 °C for 8 minutes (the solution then turns slightly blue-green), after cooling in an ice bath, the tube is left to stand in the dark for 30 min, the reading is taken with a spectrophotometer at 585 nm (Davoodi *et al*.,

2007). The quantification is done according to the equation of the followingcalibration curve Y = a X + b (mg/g of Dry Matter). That makes glucose a standard and the soluble sugar content is ultimately expressed in mg / g DM.

Dosage of Proteins

1 g of powder from the plant is taken to which 5 ml of distilled water are added, for the assay 200 µl of the extract is added to 2 ml of Bradford's reagent, the tube is shaken and left to stand for 5 minutes until 'stabilization of the color. The reading is taken by spectrophotometer at 595 nm after calibration of the apparatus with a reference solution containing 200 µl of BSA (Bovine Serum Albumin) and 2 ml of Bradford's reagent. The results are expressed in g of proteins per 100 g of Dry Matter(Saleem *et al.*, 2020).

Dosage of chlorophyllian Pigments (Arnon and Mc Kinney)

Successively put in a mortar 100 mg rounds of the leaves (cut out with a cookie cutter), a tip of a sand spatula and a tip of a spatula of calcium carbonate ($CaCo_3$) with 5 ml of 80% acetone. Grind with a pestle, 1 ml of filtrate is added 9 ml of 80% acetone and measure the optical density OD. Arnon and Mc Kinney have established systems of equations which allow the calculation of chlorophyll concentrations from absorbance measurements at 663, 645 and 460 nm of an 80% ketone extract.

Chl a = (0,0127x DO663) - (0,00269x DO645) ($mg.ml^{-1}$)

Chl b = (0,0229x DO645) - (0,00468x DO663) ($mg.ml^{-1}$)

The concentrations of chlorophylls (a and b) expressed in mg / g of fresh material are determined according to the following formulas:

Chl a (mg/l) = 12, 7 x DO663 - 2, 69 x DO645 ($mg.ml^{-1}$).

Chl b (mg/l) = 22, 9 x DO645 - 4, 68x DO663 ($mg.ml^{-1}$).

Chl a (mg/g MF) = Chl a x d x v/ (w).

Chl b (mg/g MF) = Chl b x d x v/ (w).

Cht = Ch a + Ch b.

d: dilution, v : volume of solution extracted (ml), w : mass of fresh plant material (g).

Dosage of polyphenols

Determination of the total polyphenol content): 3 g of powder from the plant are introduced into a mortar, with 150 ml of methanol-water mixture (60/40), after a maceration of about 15 min the mixture obtained is filtered through a Whatman filter paper, the aqueous phase recovered is concentrated in a rotary steamer at 45 °C. A viscous extract is thus obtained which is recovered in 3 ml of methanol. The total polyphenol content of the Galactites tomentosa plant is determined according to the method of Folin Ciocalteu (Marsoul *et al.*, 2020). 0.5 ml of the extract obtained and 0.5 ml of Folin Ciocalteu reagent are introduced into a glass tube, mixed properly for 5 minutes, 5 ml of 7% aqueous sodium carbonate solution are added and 12.5 ml of distilled water, the mixture is stirred in a vortex and stored at room temperature away from light for one hour, the absorbance is measured at 750 nm, the blank is represented by distilled water. The concentration of total polyphenols is determined by referring to the calibration curve obtained using gallic acid as a standard.

Method of Extraction

Extraction by Soxhlet

- 20 g of the dry matter are placed in the Soxhlet cartridge. - The cartridge is placed in a Soxhlet extractor (brand Gerhardt bonn App Nr. 451260). - Our extraction will take place in 3 stages with 3 solvents of different polarities: ethyl ether / chloroform / ethyl acetate. - 250 ml of ethyl ether are poured into a round ground-necked flask. - Conduct the heating T °: 60 -70 °C so as to obtain 8 distillations. - After 5 hours of extraction, the ethyl ether is evaporated off using a rotavapor (heidolph type laborota 4000) to remove the solvent. - We keep the cartridge with the plant material for the 2 solvents. - For a new cartridge, we redo the extraction using hexane.

Extraction of polyphenols by cold maceration

This is a solid-liquid extraction the solvent used in this present study is 99% pure methanol (Benakmoum *et al.*, 2008; Diallo *et al.*, 2004). This has the advantage of being easily removed under vacuum. It also gives a better extraction yield exceeding that of water(Owen & Johns, 1999). The polyphenol extraction yield also increases with the contact time. 3 g of powder from the plant are introduced into a mortar, with 150 ml of pure methanol mixture, after a maceration of approximately 48 hours the mixture obtained is filtered through a Whatman filter paper, the aqueous phase recovered is concentrated in a rotavapor at 45 ° vs. A viscous extract is thus obtained which is recovered in 3 ml of methanol.

Antibacterial activity

The antibacterial tests were carried out on strains obtained from the laboratory of medical analyzes. They were stored on sloping agar, we carried out subcultures on nutritive broth in tubes. Here is the list of bacteria used: Gram + bacteria (Staphylococcus aureus, Streptococcus pneumoniae) and Gram - bacteria (Klebsiella pneumoniae, Serratia odorifera).

The minimum inhibitory concentration (MIC)

It makes it possible to determine the smallest concentration (expressed in micrograms / ml) capable of inhibiting the growth of the bacteria considered to be the minimum inhibitory concentration or MIC. Based on concentration gradients, this is the most commonly used manual method. It is based on the fact that a disc impregnated with antibiotic in decreasing dilutions (P 1/2, 1/4, 1/8, etc.) and deposited on an HD agar previously inoculated with the bacterial suspension to be tested, will diffusing at a concentration gradient, and that the bacteria will not grow around the edges of the discs contain concentrations equal to or greater than the minimum inhibitory concentration. The process uses discs 6 millimeters in diameter, the paper of which has been impregnated with the extract of known concentration.

Operating mode

Preparation of the inoculum: Cultivate the four bacterial strains separately in petri dishes containing TSA medium (Soybean and casein peptone agar medium) at a temperature between 30-37 °C for an appropriate incubation period. for each strain. Transfer well isolated colonies with a platinum loop to sterile tubes containing non-selective broth. Preparation of the culture medium: the culture medium

consists of a layer of Mueller Hinton agar, pour a layer 2 mm thick (15 to 20 ml) distributed evenly into petri dishes and allow to solidify. Preparation of dilutions: the methanolic extract is prepared at different concentrations, there is a stock solution of 6 mg / ml from which dilutions 1/2, 1/4, 1/8, up to 1/64 are made which are dissolved in DMSO solvent(Panda *et al.*, 2017). Preparation of the discs: 5 sterile discs 6 mm in diameter are placed in each petri dish prepared previously. Antimicrobial test: the susceptibility test was performed using the disk diffusion method. Using a swab that is soaked in our already prepared microbial suspension and inoculated vertically, horizontally and around the agar(Jaberian *et al.*, 2013). Using sterile forceps and under a clean, sterilized host, the discs were placed on the surface of the inoculated medium(Silva *et al.*, 2016). Incubation: incubation takes place at 37 ° C in an oven for 24 h. the tests are repeated three times. All the work is done in front of the bunsen burner.

Results and Discussion

Phytochemical Screening

The results of the phytochemical screening are shown in table 2.

Table 2: Phytochemical Screening

Secondary metabolites	test
Alkaloids	-
Sapnosides (foam test)	++
Flavonoids	+
Tannins	++
Volatile oils	-
Quinones	+

Meaning of the symbols: (++) abundantly present, (+) presence, (-) absence Examination of the table highlights the presence and absence of these bioactive substances in our plant, we can see that saponins and tannins are very abundant, followed by other components flavonoids, quinones, and the absence of alkaloids and volatile oils. The presence of biologically active phytochemicals that act as stimulants G.R.P.I. (Abeysiri *et al.*, 2013). The phytochemical screening gives us primary information about our plants, and based on these results, we have chosen the extraction methods as an example we did not do the hydrodistillation to extract the volatile oils.

Dosages by spectrophotometer

The results of optical densities read by spectrophotometer are shown in table 3.

Table 3: Dosages by spectrophotometer

Dosage	Wavelength (nm)	Optical density
Dosage of Soluble Sugars	585 nm	0,296±0,002
Dosage of Proteins	595 nm	0,405±0,020
Dosage of Polyphenols	750 nm	0,212±0,010
Determination of Chlorophyll pigments	460 nm	0,115±0,010
	645 nm	0,097±0,010
	663 nm	0,033±0,010

We made calibration curves to determine the concentration of polyphenols (Y = 0,3971 X – 0,0131 et R^2 %= 99,75%), protein (Y = 0,0649 X – 0,0191 et R^2 %= 99,03%) and soluble sugars (Y = 39,943 X – 0,0107 et R^2 %= 98,94%). Chlorophyllian pigments are calculated by the system establish by (Arnon et Mc Kinney) Chl a = (0,0127x DO663) - (0,00269x DO645) $mg.ml^{-1}$ et Chl b = (0,0229x DO645) - (0,00468x DO663) $mg.ml^{-1}$.

The results are shown in table 4.

Table 4: Concentration of soluble sugars, proteins, polyphenols and chlorophyll pigments.

Assay by spectrophotometer	Concentration
Soluble Sugars	5,51 ± 0,04 mg/g DM
Proteins	14,86 ± 0,30 g/100g DM
Polyphenols	12,24 ± 0,10 GAE mg/g DM
Chlorophyllian pigments	1,13 ± 0,20 mg/g FM

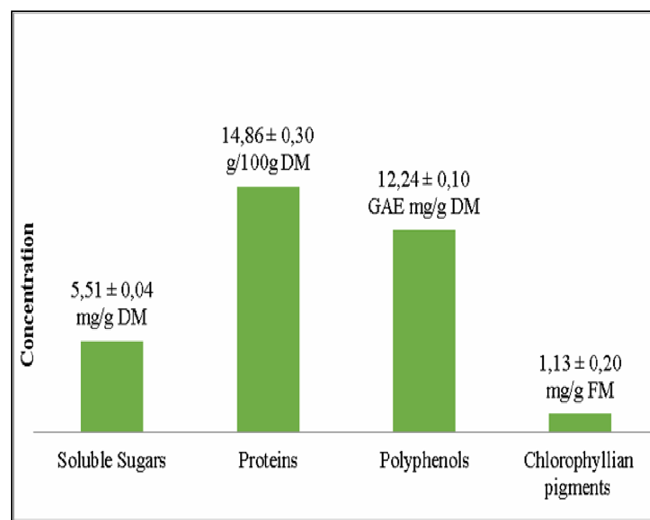

Fig. 1: Content in soluble sugars, proteins, polyphenols and chlorophyll pigments.

The results represented by figure 1, show that the proteins constitute the major component of the plant, with a concentration of 14.86 ± 0.30 g / 100g DM, this increase compared to the study by (Kolla *et al.*, 2021), confirms the nutritional value of the plant. Polyphenols are abundantly present with a concentration of 12.24 ± 0.10 GAE mg / g DM, with a somewhat low value in the study by G.R.P.I. (Abeysiri *et al.*, 2013). This shows the richness of this species in natural products and turned out to have biological activities and a concentration of 5.51 ± 0.04 mg/g DM of soluble sugars close to that of the study Quannu (Yang *et al.*, 2020). The content of soluble sugars is a key factor affecting the quality of plants. Plant proteins are made up of the same amino acids as animal proteins. In the body, proteins play essential roles: They play a structural role and participate in the renewal of muscle tissue, integuments (hair, nails, body hair), bone matrix, skin, etc. They participate in many physiological processes, forexample in the form of digestive enzymes, hemoglobin, hormones, receptors or immunoglobulins (antibodies). Distributed in the plant kingdom and in our food, known for their antioxidant properties. They are made up of a complex assembly of smaller molecules, the phenols, comprising a benzene ring and hydroxyl functions, its substances have a very important role in the life cycle of the plant. Polyphenols are becoming increasingly important, in particular thanks to their beneficial effects on health. Indeed, their role as natural antioxidants is generating increasing interest in the prevention and treatment of cancer, inflammatory, cardiovascular, and

neurodegenerative diseases. They are also used as additives for the food, pharmaceutical and cosmetic industries. For chlorophyll pigments, our plant contains 1.13 ± 0.20 mg / g FM, this is almost the same value of the results of Sathishkumar *et al.* (2012). These pigments absorb the light used for photosynthesis.

Extraction yield with Soxhlet et maceration

In order to be able to determine the best extraction solvent, that is to say the one which gives us the best yield, we proceeded to the extraction using the Soxhlet apparatus with different solvents in order to extract the lipid part and using cold maceration with methanol for the polyphenolic part. Then we calculated the yield which is the ratio of the mass of the extract obtained after evaporation of the mass of dry plant material. After 5 hours of extraction with a Soxhlet apparatus, we were able to obtain a solvent-vegetable extract mixture. We subsequently recovered our extract using a rotavapor. The extract obtained is put in a bottle covered with aluminum foil to protect it well from light, then store it in the refrigerator. *Galactites tomentosa* extract has an elastic green paste structure. The extraction yields are given in table 5.

Table 5: Solvent extraction yield

Extraction by Soxhlet				
Solvent	ethyl ether	chloroform	ethyl acetate	hexane
Yield	1,25%	0,80%	0,60%	2,00%
	Total: 2,65%			
	Extraction by maceration of polyphenols			
Solvent	methanol			
Yield	6,00%			

The results show that cold maceration with methanol gives a satisfactory yield of 6.00%. For the Soxhlet extraction we observe that the total yield of successive extractions with three different solvents is relatively better 2.65% than that obtained using hexane as the extraction solvent (yield of 2.00%).

Study of the antibacterial activity of the polyphenolic extract

The results of the antibacterial activity of the extract obtained by cold maceration using methanol as a solvent are reported in table 6.

Table 6: Antibacterial activity of extracts of *Galactites tomentosa*. Inhibitory zones in mm as a function of concentrations in mg/ml.

		Dilution of the methanolic extract						
		1	**1/2**	**1/4**	**1/8**	**1/16**	**1/32**	**1/64**
		600 mg/ml	**300 mg/ml**	**150 mg/ml**	**75 mg/ml**	**38 mg/ml**	**19 mg/ml**	**9 mg/ml**
Gram +	*Staphylococcus aureus*	17±1	14±1	11±0	8±1	-	-	-
	Streptococcus pneumoniae	28±2	17±1	15±2	13±1	10±1	-	-
Gram –	*Klebsiella pneumoniae*	35±2	28±2	20±1	15±1	12±1	-	-
	Serratia odorifera	20±2	14±1	13±1	10±1	8±1	-	-

The diameters (D) of the inhibition zone vary between 8 ± 1 mm and 35 ± 2 mm. Strongly inhibiting: D> 28 mm, moderately inhibiting: 16 mm <D <28 mm, slightly inhibiting: 10 mm <D <16 mm, non-inhibiting: D <10 mm.

In this work, values for minimum inhibitory concentration (MIC) were calculated. For Staphylococcus aureus, we obtained an MIC of 150 mg / ml for a dilution of 1/4, for Streptococcus pneumoniae the MIC is 75 mg / ml with a dilution of 1/8, for *Klebsiella pneumoniae* the MIC is 38 mg / ml with a 1/16 dilution, at the end for *Serratia odorifera* a MIC of 75 mg / ml was noted for a dilution of 1/8 (Fig. 2). Compared to the study by Rashmi (Pa & Mathew, 2012). Which revealed antibacterial activity and a potential source of antibacterial agents that could be useful for the control of infectious diseases.

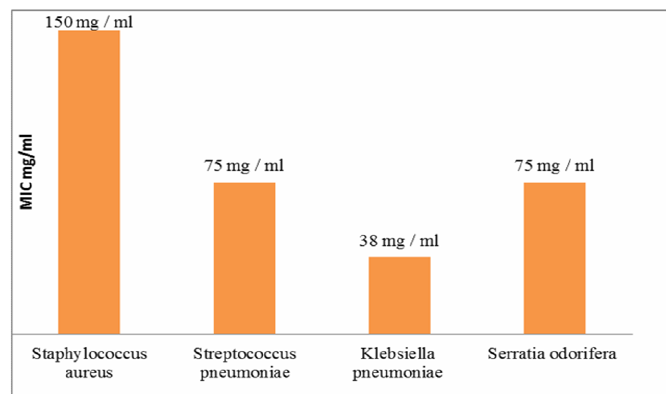

Fig. 2 : Minimum Inhibitory Concentration (MIC) as a function of Dilution

The results obtained show that the methanolic extract obtained by cold maceration has a significant inhibitory activity against bacterial growth with a different degree linked to the nature of the bacterial strains, which confirms the results of phytochemical tests.

Conclusion

This work represents a contribution to the phytochemical study of the Algerian flora plant *Galactites tomentosa* of the Asteraceae family growing in El-Tarf (EKNP). The phytochemical screening carried out revealed the existence of biologically active compounds. The presence of these compounds attributes to this species several characteristics allowing it to be used in the pharmaceutical, cosmetic and food industry sectors. The study of the antibacterial activity of the plant extract shows very promising antibacterial activity, resulting in very interesting inhibition diameters and MIC values (38.0 to 150.0 mg / ml). It would therefore be interesting to continue this experimental work, to perform an extraction on a larger mass of plant material and to proceed with the separation, purification and structural determination of the various secondary metabolites of this species by combining different methods of analysis.

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