PHYTOCHEMICAL STUDY OF MEDICINAL PLANT « *THAPSIA GARGANICA* »

F. MERATATE[1] , A. LALAOUI [2], K. REBBAS[3] , G. AICHE [3] , H. BENSEDDIK[1] , H. MERATATE[4] , S. AKKAL[5], L. DJARRI [5], I. DEMIRATAS[6]

[1]*Department of chemistry, University of M'sila, Algeria.*
[2]*University of Algiers, Algeria.*
[3*]*Department of Natural and Life Sciences, University of M'sila, Algeria.*
[4]*Department of pharmacy, Algeria..*
[5]*Chemistry Department ,University of Constantine1,25000, Constantine, Algeria*
[6]*University of Çankırı Karatekin, Faculty of Science, Department of Chemistry, Ballica Campus, 18100 Çankırı/TURKEY*
**Corresponding author : rebbas.khellaf@gmail.com ; khellaf.rebbas@univ-msila.dz*

Abstract : *The plant Thapsia garganica belongs to the family Apiaceae and is widespread in the Mediterranean basin. It is considered one of the most famous medicinal plants since ancient times. We started this work with the methanolic extraction of all the components contained in the plant in order to obtain a crude extract, which we separated with different polar solvents "petroleum ether, chloroform, and ethyl acetate and n-butanol"*
The flavonoids were evaluated by the method of aluminum chlorides $AlCl_3$, the content is estimated at 5.6009 mg EQ / g dry matter in the crude extract.
We extracted the essential oils using a Clevenger device, and then we studied the antimicrobial and antioxidant activity of all extracts and compared them among themselves.

Key words: *medicinal plants, antioxidant activity, antibacterial activity.*

1. Introduction

Medicinal plants have discovered and used in traditional medicine since prehistoric. they are a rich source of bioactive phytochemicals or vital nutrients.The widespread of plant medicine is attributed to several factors such as various claims on the efficacy or effectiveness of plant medicines, high cost and side effects of most modern drugs, and a movement toward self-medication [1].

The Apiaceae is a plant family comprising at the present time 466 genera and about 3800 species [2]. Commonly known as the celery, carrot or parsley family consists of dicotyledonous plants characterized in particular by their typical inflorescence [3].

Thapsia is a genus belonging to the Apiaceae family .they are flowering plants with 41 species native to Africa, Asia and Europe. *Thapsia garganica* is a widespread species in Algeria, best known for the use of its roots in cooking and traditional medicine [4]. It is a wide spread species in the Mediterranean basin, which inhabits roadsides and fields [4].

This perennial plant has a striated, glabrous stem, branched in its upper part, reaching 0.90 to 1.40 m in height. The leaves are green, hairless. The primordial leaves are small, elliptical and entire, the following ones are palmatilobed. The leaves at the base of the stem are large, 2-3 pinnately shaped, the upper ones are reduced to a wide sheath. The root is large, blackish on the outside, white on the inside.

The inflorescence is a large, compound umbel with 15-20 rays, bearing yellow flowers. The involucre and involucelle are absent. Umbellules are globular in shape.The fruit is elliptical, dorsally compressed, 10-15 by 20-25 mm, with notches more or less wide at the top and at the base.

Lateral wings very developed, shiny, straw yellow, finely streaked [5,6]. *Thapsia garganica* is a Mediterranean plant. It is present in Morocco, Algeria, Tunisia and Libya [7], but also in Turkey, Spain, Portugal, Italy and Greece.

Thapsia garganica it is a perennial plant, with an erect, branching flowering stem, reaching a height of about 1.50 meters. It has large leaves in tufts, very indented, linearly divided with the stem. The flowers are small, yellowish, arranged in large, nearly spherical umblas.

The fruits are oval, reach more than 2 cm in length, wide-winged. The roots are rhizome-shaped Flowering occurs between April and July. Extracts from the roots of *Thapsia garganica* used for the treatment of lung diseases, colds and relief of rheumatic pain [4].

They contain strong irritants to the skin, constituting an important cause of contact dermatitis, which manifests itself by erythema, itching and the formation of small vesicles.

The main active compounds in the majority of root extracts responsible for these effects identified by Christensen & al. (1982) [8] as sesquiterpenes known as thapsigargine and thapsigargicine.

A study on the cytotoxicity of tapsygargin and phenylpropanoid esters isolated from *Thapsia garganica* flowers conducted by [9].

The tests done on leukemia, and breast cancer cell. IC50s revealed that thapsigargine has higher cytotoxic potentials compared to phenylpropanoid esters.

Fig. 1. *Illustration of Thapsia garganica (left, plant habit and right, inflorescences), photos K. Rebbas.*

2. Vegetable material

The aerial parts of the studied plant collected in June (Bejaia, Algeria) and identified by Pr K. Rebbas, a botanist from the University of M'sila, The choice of this plant is based on its use in traditional Algerian medicine. After harvesting, the collected plant material dried at room temperature and protected from light.

The resulting dry matter reduced to powder using an electric grinder. The latter kept in closed glass jars and stored away from light.

3. Extraction of essential oil

The aerial parts of the plant aresubjected to hydrodistillation (for 3 hours) using a "clevenger" type extraction device**,** the operation consists of immersing a quantity of the plant mass (100g) in a glass flask. (6 liter) containing a sufficient quantity of distilled water without completely filling the flask.

The mixture is brought to a boil using a heating mantle.The vapors charged with the essential oil pass through the refrigerant where the condensation will take place. Due to the difference in density, the oil floats on the surface of water and it is recovered, and then dried by a desiccant (sodium sulfate) to remove the little water likely to have been retained in the oil.

The essential oils obtained are stored in bottles protected from light and at a temperature of 4°C.

4. Preparation of the extracts

Thapsia garganica powder was soaked in 70%aqueous-methanol. The extract was filtered. This procedure was repeated twice on the residue using 80%, 90% aqueous –methanol respectively to obtain the last filtrate. The first and the last filtrates were combined then the methanol was removed under reduced pressure on a rotavapor below 45°C.

Crude extract (CrE) was subjected to fractionation using liquid-liquid extraction. CrE was successively extracted with different solvents of increasing polarity: petroleum ether for defatting,dichloromethane for aglycone flavonoids extraction and n-butanol for glycoside flavonoids extraction.

The obtained organic layer of each partition was evaporated under reduced pressure on a rotavapor below 45°C to dryness and to afford petroleum ether, dichloromethane and n-butanolic fractions coded as PE, DE and BE, respectively.

5. Determination of the total flavonoid content

The quantification of flavonoids carried out by a method based on the formation of complexes between phenolic compounds and aluminum trichloride [10]. The content of total flavonoids in the extracts of the plant studied was determined according to the method described by Ayad & al. (2017) [11]. One milliliter of each extract dissolved in methanol (1mg / ml) added to 1ml of $AlCl_3$ solution (2% in methanol).

The mixture stirred vigorously and the absorbance read after 10 minutes at 430 nm. A calibration curve produced by quercetin at different concentrations, performed under the same operating conditions as the samples.

6. Antioxidant activity in vitro

Antioxidants sometimes called "free radical scavengers" are all molecules capable of inhibiting production in low doses, limiting the proliferation or destruction of reactive oxygen species. They prevent cell damage from highly reactive and unstable molecules called "free radicals" [12, 13].

The balance between antioxidants and free radicals in our bodies is important for health. If not controlled, free radicals lead to cell damage associated with a variety of chronic diseases. The sources of antioxidants can be natural or artificial. The body also produces some antioxidants known as endogenous antioxidants in contrast to antioxidants that come from outside the body called exogenous [12].

Indeed, DPPH characterized by its ability to produce stable free radicals; this stability is due to the delocalization of free electrons within the molecule**.** The presence of these DPPH • radicals gives rise to a dark violet color in the solution. has been proven that the reduction of DPPH • radicals by an agent antioxidant causes discoloration of the solution, and the color change can be followed spectrophotometrically at 517nm and in this way the antioxidant potential of a substance or a plant extract can be determined [14].

DPPH 2,2 – Diphenyl – 1 – picrylhydrazyl ($C_{18}H_{12}N_5O_6$; Mr: 394.33), is dissolved in absolute methanol (4 mg / 100ml).For the test, the samples were prepared by dissolving in absolute methanol. For all extracts, solutions in absolute methanol were prepared. These solutions, called stock solutions, will then undergo dilutions in order to have different concentration.

The protocol used for the evaluation of the scavenger effect of plant extracts against the DPPH radical is that of "Cuendet" with a small modification [15].

50 µl of the test solution are introduced into dry and sterile tubes, 1250 µl of the DPPH solution are added. After vortexing, the tubes placed in the dark at room temperature for 30 minutes. For each concentration, the test repeated 3 times. The reading taken by measuring the absorbance at 517 nm by a spectrophotometer. The negative control is composed of 1250 µl of the methanoic solution with DPPH and 50 µl of methanol.

7. Expression of results

To obtain the effective concentration, which reduces the initial concentration of DPPH by 50%, the results expressed as antioxidant activity. The antioxidant activity, which expresses the capacity to scavenge the free radical, estimated by the percentage of discoloration of the DPPH in solution in methanol. The "AA%" antioxidant activity given by the following formula:

$$\mathbf{AA\ \%} = \mathbf{100} - \{[(\mathbf{Abs_{test}} - \mathbf{Abs_{blanc}}) \times \mathbf{100}]/\mathbf{Abs_{control}}\}$$

$$\mathbf{Inhibition\ \%} = (\mathbf{Abs_{control}} - \mathbf{Abs_{test}})/\mathbf{Abs_{control}} \times \mathbf{100}$$

AA: Antioxidant activity.
Abs: Absorbance at the wavelength of 517 nm.

The results expressed as the mean of two measurements ± standard deviation. The IC 50 value was determined for each extract, is define as the concentration of the substrate, which causes the loss of 50% of the activity of DPPH (color).

8. Antibacterial activity

The aim of this work is to evaluate the antibacterial activity of the essential oil and the other four extracts (n-butanol extract, ethyl acetate extract, chloroform extract and petroleum ether extract) of *Thapsia garganica* against bacteria.

a. Diffusion method in solid medium

The study carried out by the diffusion method, which is initially designed for antibiotics (antibiogram), but substituting the antibiotic discs with others impregnated with (n-butanol extract, extract of acetate ethyl, chloroform extract, petroleum ether extract) of *Thapsia garganica*. This method consists of depositing sterile Wattman paper discs N° 3 and 6 mm in diameter impregnated with 10 µl of each extract with different concentrations, the discs placed at the surface of the petri dish in the presence of discs soaked with an aqueous solution (negative control) placed at the agars seeded with the germ to be tested and to measure the diameters of inhibition in millimeters (mm) after incubation.

b. Preparation of solutions

We dissolved each of the essential oil extract and four other organic extracts so that:

- The different organic extracts of the studied plants were dissolved in DMSO and dilutions were made to obtain concentrations of 0.2 g/ml for each tested extract.
- The essential oil extract obtained from distillation was dissolved in DMSO in order to obtain a concentration of 0.1mg/ml.

c. Microbial strains used

The strains used to detect the antibacterial activity of *T. garganica* extracts belong to four genera of microorganisms, which are reference strains of the American Type Culture Collection (ATCC), these are: *Staphylococcus aureus (ATCC 25293), Escherichia coli (ATCC 25922), Pseudomonas aeruginosa (ATCC 27853), Enterococcus(ATCC 51299).*

Table 1: *Microbial strains used*

Gram+	*Gram-*
• *Staphylococcus aureus (ATCC 25293)* • *Enterococcus (ATCC 51299)*	• *Escherichia coli (ATCC 25922).* • *Pseudomonas aeruginosa (ATCC 27853).*

d. Preparation of the inoculum

First, a bacterial suspension is prepared from a pure and young culture (18 hours old). This inoculum used to inoculate Mueller Hinton agars poured into petri dishes to a thickness of 4 mm and then dried in an oven at 37°C before use. Inoculation carried out by swabbing, from the freshly prepared inoculum.

It consists of dipping a sterile cotton swab in the suspension then rubbing it, after having wrung it inside the tube, three times over the entire agar surface so the inoculation carried out by swabbing, from the inoculum to form tight streaks, rotating the dish approximately 60° after each application to obtain an even distribution of the inoculum.

Pre-sterilized watman paper discs 6 mm in diameter placed on the surface of the inoculated agar after having been loaded with 10 µl of extract. After 24 hours of incubation at 37 ° C, the diameter of inhibition is measured.

9. Results and discussions

9.1. Total flavonoid content results

The determination of the flavonoids was carried out according to the trichloride method of aluminum $AlCl_3$ and the standard was quercetin, The flavonoid content was calculated for the crude extract which is expressed in µg EQ / mg of extract.

Before proceeding to the determination of the content of flavonoids we have established a calibration curve using quercetin as a reference compound. The flavonoids were evaluated by the method of aluminum chlorides $AlCl_3$, the content is estimated at 5.6009 mg EQ / g dry matter in the crude extract.

9.2. Result of antioxidant activity evaluation in vitro

- Trapping of the 2-diphenyl-picrylhydrazyl radical (DPPH) :

The DPPH test has attracted a lot of attention due to its speed, sensitivity and reproducibility. The results obtained in this study reveal a proportional relationship between the concentration of petroleum ether / acetate ethyl extracts as well as the crude extract and the BHT standard with the percentage inhibition of the DPPH radical.

The IC 50 of the different extracts are compared with the BHT standard. The IC50s in the order of antioxidant power were: petroleum ether (42,2938144 mg / ml) > acetate ethyl (0,67893784mg / ml> BHT (0.0059 mg / ml).

Depending on the results we have obtained, we can predict that flavonoids are first class antioxidant agents.

9.3. Antibacterial activity

The results regarding the Antibacterial activity of the essential oil and the four extracts petroleum ether, chloroform, acetateethyl, n-butanol of "*Thapsia garganica*" are indicated in Table 2. The results obtained from disc diffusion method indicated that the essential oil and the four extracts didn't show any Antibacterial activity against all microorganisms tested.

The diameters of inhibition of *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus (*ATCC *25293), Escherichia coli (*ATCC *25922), Enterococcus(*ATCC *51299)*, was 6 mm and this indicates the insensitivity of the bacteria against both the essential oil and the extracts.

Table 2. *Antibacterial activity of essential oil and the extracts of Thapsia garganica*

Bacterial strain	Diameters of inhibition zones (mm)				
	N-butanolextract (NB)	Acetate ethyl extract (AC)	Chloroform extract (CH)	Petroleum ether extract (PE)	Essential oil extract
Escherichia coli (ATCC 25922	6	6	6	6	6
Staphylococcus aureus (ATCC 25293),	6	6	6	6	6
Pseudomonas aeruginosa (ATCC 27853)	6	6	6	6	6
Enterococcus	6	6	6	6	6

10. Conclusion

Thapsia garganica is one of the most important plants in traditional and modern medicine, it belongs to Apiaceae family. It was devoted to studying the antibacterial and antioxidant properties of this specie, as well as the discover of its polyphenolic content.

Quantitative determination of total flavonoids by the alimunium chloride $AlCl_3$reagent revealed that *T. garganica* is rich in flavonoids.

The study of the antibacterial activity by the method of diffusion on agar medium showed that the plant did not exhibit an antibacterial activity on most of the strains used.

The study of the antioxidant activity showed that the plant rich of antioxidant elements, it could be used as a source of natural polyphenols and antioxidants.

References

1. Bandaranayake, W M. : Quality control, screening, toxicity and regulation of herbal drugs Mod. Phytomedicine, 2006, 1, pp. 25-57;
2. Plunkett, G.M., Pimenov, M.G., Reduron, J.-P., Kljuykov, E.V., Lee, B.-Y., van Wyk, B.-E., Tilney, P.M., Watson, M.F., Ostroumova, T.A., Spalik, K., et al. , Apiaceae. In: Kadereit, J. and Bittrich, V. (eds) The Families and Genera of Vascular Plants. Vol. 15. Flowering Plants. Eudicots. Springer, New York , 2018, pp. 9–20;

3. Magee, A. R., Calviño, C. I., Liu, M., Downie, S. R., Tilney, P. M., & Wyk, B. E. V. : New tribal delimitations for the early diverging lineages of Apiaceae subfamily Apioideae. Taxon, 2010, 59(2), 567-580;
4. Gómez, F. L. M. : Síntesis d'análogos de las tapsigarginas. Mémoire en vue d'obtention du grade de doctorat en chimie. Université de Cádiz. puerto real. Espagne, 2007;
5. Quezel, P. et Santa, S. : La nouvelle Flore de l'Algérie et des régions désertiques méridionales, T1et 2, 1962-1963;
6. Pottier-Alapetite, G. : Flore de la Tunisie, vol. 1, Tunis, Ministère de l'Enseignement supérieur et de la Recherche scientifique et ministère de l'Agriculture, 1979, 612 p;
7. Dobignard, A., Chatelain, C., Fischer, M., Orso, J., & Jeanmonod, D. : Index synonymique de la flore d'Afrique du Nord. Conservatoire et Jardin botaniques, 2010;
8. Christensen, S. B., Larsen, I. K. & Rasmussen, U. : Thapsigargin and Thapsigargicin: Two Histamine Liberating Sesquiterpene Lactones from Thapsia garganica. X-ray Analysis of the 7, 11-Epoxide of Thapsigargin. J. Org. Chem. 1982, 47 (4): 649-652;
9. Liu H., Jensen, K. G., Linh My Tran, L. M., Chen, M., Zhai, L., Olsen, C. E., Sohoel, H., Denmeade, S. R., Isaacs, J.T. & Christensen, S.B. : Cytotoxic phenylpropanoids and an additional thapsigargin analogue isolated from *Thapsia garganica*. Phytochemistry, 2006, 67 : 2651–2658;
10. Ribéreau-Gayon, P. : Composés phénoliques des végétaux. Edition dunod, 1968, pp 105-133;
11. Ayad, R.; Cakmak, Y.S.; Ozusaglam, M.A.; Medjroubi, K.; Akkal, S. : In vitro antioxidant, 2017;
12. Shalaby, E., & Catala, A. : Antioxidants. BoD–Books on Demand, 2019;
13. Favier, A. : Le stress oxydant. Intérêt conceptuel et expérimental dans la compréhension des mécanismes des maladies et potentiel thérapeutique. Actualité en chimie, 2003, pp 108-115;
14. Molyneux, P. : The use of the stable free radical diphenylpicryl-hydrazyl (DPPH) for estimating antioxidant activity.SongklanakarinJornal of Science and Technologie, 2004, 26, 211–219;
15. Cuendet, M. Hostettmann, K. Dyatmiko, W. Potterat, O. : Iridoid glucosides with free radical scavenging propreties from Fagraea blumei.Helvitica Chimica Acta.1997. 80: 1144 -1152.