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Der Pharma Chemica, 2014, 6(1):443-447
(<http://derpharmachemica.com/archive.html>)



ISSN 0975-413X
CODEN (USA): PCHHAX

Preliminary phytochemical study of leaves and roots of *Fredolia aretioides*, endemic plant of Algeria

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ABSTRACT

The majority of the current drugs come from the medicinal plants. A better valorization of the Algerian medicinal plants passes by their scientific studies. This work, returns within the framework of the research program of LAPSAB laboratory, intended for the valorization of the local flora. It consists of the discovery of new compounds with therapeutic outlets. For that, we were interested in the study of the extracts of *Fredolia aretioides*. The current studies relating to the secondary metabolites, obviously stick to explore their pharmacological activities. Our work aims at making a phytochemical screening of the two parts (Sheet-stems and roots) and to determine the amount of their polyphenols. Phytochemical tests on the two parts of the plant have shown the presence of alkaloids, tannins, saponins and reducing compounds. Coumarins are present only in the aerial part. The content of total phenols varies between the two parties. The methanol extract of the root contains a highest content of total phenols (917.05 Mg GAE/100g).

Key words: *Fredolia aretioides*, Secondary Metabolites, Polyphenols, Extracts.

INTRODUCTION

The share of unexplored plants at the same time in chemistry and biology is still immense. What offers the hope to still discover treatments for devastating diseases and to propose not very expensive therapeutic alternatives, with less undesirable effects. The current studies relating to the secondary metabolites, obviously stick to more explore their pharmacological activities [23, 6].

Algerian flora abounds with many species of plants, that did not yet studied, but equipped with real pharmacological properties [11, 4, 10]. The total and perfect control of the various properties of these plants, passes by the determination of the whole physicochemical groups able to generate one or more pharmacological effects, is today an objective which occupies an order of first place [1].

This is why, we were interested in making a phytochemical study of the plant *Fredolia aretioides* of the area of Béchar (Algeria). *Fredolia aretioides* endemic species of Algerian and south-eastern south-west Moroccan belongs to the family of *chenopodiacaes*, a largely widespread family in the moderate saline habitats, in particular in the littoral areas of the Mediterranean, the arid steppes and the deserts [17]. *Fredolia aretioides* has the shape of a brush of 1 m in height. The branches are very compact with sand. The small sheets and coriaces are of color blue-green and of fleshy forms, not exceeding 5 Mettres. The fruit is an akene surrounded by small transparent wings of the persistent perianth. [20,18,2].

The present study aims the valorization of the local flora in order to develop new active or principles compounds with therapeutic effects. For that, we envisage making a phytochemical study of three extracts with

increasing polarity (ether diethylic, ethanolic and aqueous) of the roots and leaves of *Fredolia aretioides* which contains the phytochemical tests and determination of total polyphenols. It will involve of characterizing the chemical groups which will allow explaining the therapeutic effects off the tested seedlings.

MATERIALS AND METHODS

2.1 Plant material

Our phytochemical study required a vegetable material represented by the roots and the leaves of *Fredolia aretioides* collected in December 2011, in the area of Bechar (Algeria). The plant used was identified within the laboratory of vegetable ecology of the university Abou bekr Belkaïd, Tlemcen. The dried roots and leaves were pulverized and used for the preparation of the various extracts.

2.2 Phytochemical Study:

- The phytochemical examination is necessary to identify the major families of compounds existing in both parts of our plant. We characterized the presence of these secondary metabolites by preparing three extracts of increasing polarity (diethyl ether, ethanol and water) to 10% in heating and continued stirring for 30 minutes and using the techniques described in the works of (Dohou and *al*, 2003), (Karumi and *al*, 2004), (Ciulel, 1982), (Trease and Evans, 1987) and (Benmehdi, 2000).
- The detection of the saponosides is carried out by adding 1mL of distilled water to 2mL of each extract for both parts (leaves and roots). Then the solution is agitated during 1 minute. The presence of saponins was confirmed by the appearance of foam than 1 cm height that persists for 15min.
- The alkaloids have been characterized from Burchard reagent. Six (6) mL of each solution were evaporated to dryness. The residue is taken again by 6 mL of alcohol with 60°. The addition of 2 drops of reagent Burchard on alcoholic solution caused a reddish-brown precipitate and indicates a positive reaction.
- The reaction of ferric chloride (FeCl₃) was used to characterize the tannins. To 2 mL of extracts were added 2 or 3 drops of the solution of FeCl₃ 1%. A positive test is indicated by the appearance of a blue-black color (gallic tannins) or blue-green (tannins cathechics).
- For the detection of flavonoids, 2mL of plant extracts are treated with a few drops of HCl 37%, and 0.5 g of magnesium turnings Mg⁺⁺. The positive test is marked by appearance of pink or red color which characterizes flavonoids.
- Several reducing compounds can be highlighted. They are the oses, the holosides and the mucilages. Their detection consists in treating 1mL of extract with 2mL of distilled water and 2mL of Fehling's solution (1 mL of liquor Fehling A + 1mL of liquor Fehling B) and heat the tubes in a water bath at 40°C. A positive test is indicated by the formation of a brick-red precipitate.
- The coumarins have been studied using the technique described by **Bruneton, 1999**. 5mL of extract of 10% of each part of the plant are evaporated to dryness. The resulting residue was solubilized in hot water. A volume of the aqueous phase was added with a solution of "NH₄OH" at 10% and another volume is kept as a control. The appearance of fluorescence after UV observation indicates the presence of coumarin.

2.3. Determination of total polyphenols:

The extract water / methanol (48 hours maceration and evaporation to dryness) was solubilized in methanol at a concentration of 1 g / L for the determination of total polyphenols. The rate of total polyphenols of roots and leaves, is determined spectrophotometrically by using the Folin Ciocalteu. The produced color, whose absorption maximum is between 700 and 760 nm, is proportional to the amount of polyphenols present in the plant extracts [5]. The dosage of these polyphenols is performed according to the method described by Vermerius and Nicholson (2006). 0.1 mL of the sample is mixed with 2 mL of a solution of sodium carbonate 2%. After agitation and incubation for 5 minutes, 100 µL of Folin Ciocalteu 1N are added and after 30 minutes of incubation at room temperature, the DO reading is made at 700 nm against a white. A calibration curve is performed in parallel under the same experimental conditions by using the gallic acid like controls positive with final concentrations going from 0,1 to 1 mg/mL in increments of 0.1.

RESULTS AND DISCUSSION

3.1. Phytochemical study:

▪ Phytochemical tests:

Phytochemical tests consist of detecting of the various families of compounds existing in the studied part of the plant by qualitative reactions of characterization. These reactions are based on phenomena of precipitation or coloring by reagents specific to each family of compounds. The results of phytochemical tests on the two parts (leaves and roots) of the studied plant "*Fredolia aretioides*" exhausted by water, ethanol or ether diethylic are given in **table N°1**. In the leaves and the roots of this plant, the research of alkaloids, tannins, compounds

reducing and saponosides was positive. The polar aqueous extract of the roots shows a presence of the flavonoïds more important than the apolar extracts; this can be attributed to the difference in the degree of polarity of the flavonoïds whose polar flavonoïds represent the highest fraction. Tannins are present with an important intensity in the two extracts: aqueous and ethanolic. They are Gallic tannins. The saponosides are strongly present, in each part of the plant with foaming pipes exceeding 3cm in height. The appearance of fluorescence under a light UV indicates the presence of coumarins only in the roots of *Fredolia aretioides* but with a low intensity. The presence of alkaloids was more important in the roots compared to the leaves. It was confirmed by a brown precipitation in contact with the reagent of Wagner. We also note the presence of the reducing compounds with a low intensity in both parts of *Fredolia aretioides*. The use of different solvents of different polarity, is used to separate compounds based on their solubility in the extraction solvent. This extraction method performed under continuous stirring, and short-term, is used to extract the maximum amount of bioactive components and prevent their modification or probable denaturation [12].

N°1 table: Results of the reactions characteristic of different chemical groups looked in different extracts *Fredolia aretioides*:

Classes searched	Aqueous extract		Ethanolic extract		Diethy ether extract	
	Leaves	Roots	Leaves	Roots	Leaves	Roots
Alkaloids	++	+++	++	++	-	++
Flavonoids	-	+	-	-	-	-
Tannins	+++	+++	+++	+++	+	+
Coumarins	+	-	+	-	+	-
Saponins	+++	+++	-	-	-	-
Reducing compound	++	++	-	-	-	-

▪ Determination of total polyphenols:

The quantitative estimation of total polyphenols was carried out by using the method of Folin-Ciocalteu reagent and the gallic acid was used as standard [15]. Based on the absorbance values of the two solutions of extracts, having reacted with the reagent of Folin Ciocalteu and compared with the standard solution in equivalence of gallic acid, the results of the colorimetric analysis of the total phenolic compounds are represented in **Figure N°1 and N°2**. The results are expressed as mg gallic acid equivalent per 100 grams of dry plant material (Mg GAE / g), using the linear regression equation of the calibration graph traced of acid Gallic.

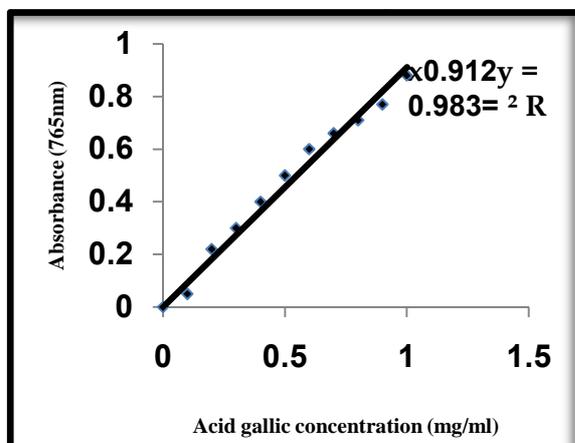


Figure N_1: Calibration graph of acid Gallic for the determination of total polyphenols

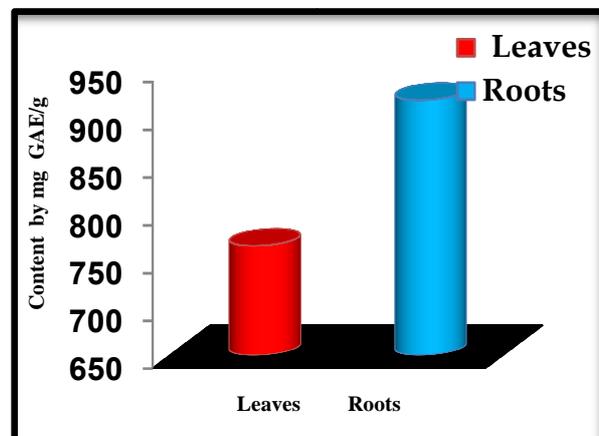


Figure N°2: Levels of total polyphenols in both parts of the study plant.

In both parts of the studied plant, we noticed variability in levels of polyphenols. The part with the highest content is the root of the order of 917.05 ± 0.83 Mg GAE/g followed by the leaves with a content of 764.54 ± 0.55 Mg GAE/g.

A study made by Rached and *et al*, (2010) shows that the content of total phenols in the roots of *Fredolia aretioides* is about $110,92 \pm 6.34$ Mg GAE/g with a negative content in the leaves. This rate is significantly lower and differs from our results which are very high in both parts of the plant. This may be due to the harvest period, which was made during the month of June for studies of Rached and *al.*, (2010), which is different from the harvest period of our plant (December).

The polyphenols content differs qualitatively and quantitatively from one plant to another, this can be attributed to several factors:

- Climatic and environmental Factors: the geographical area, dryness, ground, aggressions and diseases... etc [9].
- The genetic heritage, the period of harvest and the developmental stage of plant [16].
- The method of extraction and quantification method can also influence the estimation of the content of total phenols [14].

CONCLUSION

- In the pharmaceutical industry, knowing that the antioxidants contribute significantly to the disease prevention, the development of new synthetic methodologies and preparation of molecules for therapeutic use is a major objective and a permanent concern for many researchers. In this context, we are interested in the phytochemical study of the different extracts of *Fredolia aretioides* harvested area Bechar. The phytochemical screening carried out by reactions characterization revealed the richness of our plant on alkaloids, tannins and saponins in both parts of our planet, with a low presence of other secondary compounds such as coumarins, flavonoids and reducing compounds.
- Quantitative analysis of extracts of *Fredolia aretioides* is represented by a spectral proportioning of the total phenols which was variable between the two parts of the plant. The highest content of polyphenols is found in the roots. These important results reflect the richness of each part of the plant in secondary compounds.
- Thus, our work has allowed us to confirm the traditional uses of *Fredolia aretioides*. According to the results obtained in this study, we can say that this analysis will find an important application in the drug company as it can also be useful in food industry.

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