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ORIGINAL ARTICLE

Study of the Biological Activities of the Methanolic Extract of Globularia Alypum l from Algeria

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ABSTRACT

Medicinal plants are a very important therapeutic drug source in terms of medicinal use as they contain various secondary metabolites and bioactive compounds which procure to them several therapeutic potentials.

The present research is aimed to study some biological activities of the methanol extract of Gloubular alypum maerial part , all the preliminary tests to be carried out for the quantitative metabolites determination of the used plant which have been confirmed the presence of many different concentration of flavonoids, polyphenols and flavanols and to evaluate different antioxidant activity busing two parameters DPPH and ferric potential FRAP and to test the inflammatory effect in vivo.

The results obtained showed that the extract has an important antioxidant activity defined by 0,153. 0,116. 0,110(µg/ml). and significant anti-inflammatory activity.etalon at the following concentrations 0.23. 0.40. 0.34 (µg/ml).

Gloubulariaalypum have significant therapeutic activities based on the present results obtained in current research and comparing to previous studiesso this what explain its utilization on alternative medicine and push us for anterior research.

Keywords: anti-inflammatory activity, Biological activities, Frap, Globularia alypum , phytochemical screening

INTRODUCTION

For a long time, natural remedies and especially medicinal plants were the principals of healing , if not the only, recourse of medicine, however, despite the development of the pharmaceutical industry that has enabled modern medicine to treat a large number of diseases that were often be fatal, the medicinal plants and natural remedies that could be the origin of many pharmaceutical drugs were not totally abandoned and people never stopped using traditional medicine, which led to keeping alive the therapeutic traditions left by our ancestors. The search for new substances of plant origin useful for medicine can be done in several ways: One of them is based on the study of the traditional use of plants by the peoples of the different regions of the world, starting especially with those closest to us both geographically and culturally. This information is followed by the identification of the plant material and its harvest. In a third step, the material, well determined, is subjected to chemical and biological analysis. Another approach is to collect species-specific phytochemical data and try to extend investigations to neighboring species or related genera. For example, we know from the literature that compounds are rich in sesquiterpene lactone derivatives and flavonoids, we will then be more readily interested in species belonging to this family.

Globularia alypum L is a plant native to southern Europe around the Mediterranean to Greece, North Africa (Algeria, Morocco to the Sahara) and Asia Minor (Egypt, Arabia) in forests, in rocky terrain (Beniston 1984).

Globularia alypum has been traditionally used in many medicinal fields, as a scarring, antimycosic, diuretic, astringent, antiseptic (Bellakhdar et al.1991), anti-hypertensive and hypoglycemic (Jouad et al. 2002);(Sezik et al. 1991) The aerial part (especially leaves and flowers) is used to treat many diseases such as kidney disease, cardiovascular disease, it is also used against ulcers of the stomach, rectum, colon, liver and esophagus (Djeridane 2006). The leaves of the plant are used as laxative, purgative, stomachitis and sudorific (Bellakhdar et al 1991). It reduces histamine and serotonin in vitro (Bello et al 2005).

The present study is aimed for the valorization of Globularia Alypum by testing some important biological activity in vitro and dosing phytochemical components.

MATERIALS AND METHODS

Collection of plant materials: In this research the plant material is represented by Globularia Alypum L areal part,the present plant was collected in April 2021 in the Ariss region in Batna city, Algeria 05000, and identified at LBMBPC laboratory, university of Batna 2.

Plant leaves were dried in shade at room temperature before grinding and conservation.

Extraction of phytochemicals: Dried powder was extracted in several steps using different solvents such methanol,chloroform, petroleum and water , the obtained extract was evaporated with rotavapor BUSHI 1235 , Once the extract is obtained, it is kept in a glass bottle wrapped in aluminum foil at a specific temperature.

Qualitative test of phytochemicals: All chemical test for screening of alkaloid, terpenoids, flavonoids, glycosides, tannins and saponins in aerial part extract were checked with the general standard methods (Fransworth 1996; Harborne 1998).

Quantitative estimation of phytochemicals

Determination of polyphenols: The five extracts (200 µl) were mixed with 1 ml of Folin-Ciocalteu reagent diluted 10 times and 2 ml of H2O, and incubated at room temperature for 4 minutes. After 0.8 ml of 7.5% sodium bicarbonate was added to the mixture, the total polyphenols were determined after 2 hours of incubation at room temperature. The resulting blue absorptive was measured at λmax = 765 nanometres using a UV-VIS Shi-madzu spectrophotometer. The quantification was done for the standard gallic acid curve. (William-Koh 2006).

Determination of total flavonoids: 0.5 ml of each extract or standard (prepared in ethanol) are added to 0.5 ml of the AlCl3 solution (2 % prepared in ethanol). After one hour of incubation in darkness (Appendix VI), absorbance was measured at λ = 420 nm each reading is repeated trios times. (Djeridane et al.2006).

Determination of total flavonols: One ml of AlCl3 solution (2%) and 1.5 ml of sodium acetate (50 g/l) are added to 1 ml of each extract, incubation takes place for two and a half hours in the dark. The appearance of dark yellow indicates the presence of flavonols (Appendix VII). The absorbance of each solution was measured at λ = 440 nm. A calibration curve was carried out in parallel under the same operating conditions using quercetin at different concentrations (0.01 to 0.1 mg/ml). The results are expressed as milligram equivalents of rcetin per gram dry matter (mg EQ/g). (Kosalec et al. 2004).

In vitro tests for antioxidant activity DPPH assay: To study the anti-radical activity of the different extracts, we opted for the method that uses DPPH (diphenyl picryl-hydrayl) as a relatively stable free radical, according to the protocol described by (Mansouri et al. 2005).The DPPH solution is prepared by solubilizing 2.4 mg of DPPH in 100 ml of methanol. 25 µL of the extract or standard solutions (quercetin, TBHQ) are added to 975 µL DPPH, the mixture is left in the dark for 30 min and the discolouration from the negative control containing only the DPPH solution is measured at 517 nm.

The reducing antioxidant capacity of ferric (FRAP): The reducing power of the various extracts is estimated by applying the method of (Yildirin et al. 2001)

A volume of 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml potassium ferricyanide (C6N6Fe6K3) (1%) is added to 1 ml of each extract (at different concentrations). 2.5 ml trichloroacetic acid (C2HCl3O2) (10%) is added to the mixture after incubation 20 min at 50°C. After centrifugation at 3000 rpm for 10 min, 2.5 ml of the supernatant is mixed with 2.5 ml of distilled water and 0.5 ml of ferric chloride (FeCl3) (0.1%). The absorbance is measured at 700nm.

Anti-inflammatory activity in vitro: The anti-inflammatory activity of extracts of our plant in vitro was determined by the method of (Sangita et al. 2012). The principle consists in the inhibition of denaturation of BSA caused by heat (72°C) by the extracts.

Preparation of a range of a solution, test solution (Ts) consisting of 0.2 ml of the solution of bovine albumin serum (BSA) 0.2%, and extracts from the plant with concentrations 800, 400, 200, 100 and 50 ml. Are prepared from a stock solution of 10 000 ppm. The test solution (Tc) containing 1 ml of the BSA solution and 1 ml of the solvent. The control solution product (pc) composed of 1 ml of tris-Hcl and 1 ml of plant extracts with 800, 400, 200, 100 and 50 ml. The standard test solution (ss) consists of 1 ml of 0.2% BSA aqueous solution and 1 ml of the standard diclofenac sodium solution with concentrations 800, 400, 200, 100 and 50 ml. All the above solutions were adjusted to pH 6.6 by a solution of HCl (3N), the samples were incubated at 37°C for 15 min, then in a 72°C water bath for 5 min, after cooling the turbidity is measured at 660 nm in a spectrophotometer.

RESULTS AND DISCUSSION

Phytochemical tests: The experimental results of our motioned extracts in the following **table 1** show the presence or absence of certain chemical groups. These tests are related to the intensity of the precipitate, turbidity or staining, which are proportional to the quantity of the desired substance.

Table 1: Concentration of metabolites in the extract

M S	Extrait méthanolique.
Les Polyphenol	+
Les tanins	++++
Les flavonoïdes	++++++
Les Tèrpenoides	+++

In our work, the total polyphenols content of the ethanol extract 80% was determined by the colorimetric method of Folin-Ciocalteu, whose result was 240 6.20mg gallic acid equivalent/g dry matter (mg Eq C/g M.S).

A study conducted by (Khlifi et al. 2011) shows that the methanol extract of Globularia alypum has a value of 116.89 2.80g gallic acid equivalent/kg dry matter (g Eq AG/kg M.S) followed by acetone extract with a content of 109,46 1.1g Eq AG/kg M.S which are much lower than the one recorded by our study, of which they present almost half Also comparing with the work done by (Djeridane et al. 2006) using methanol as an extraction solvent, the results show that the total phenol content in Globularia leaves is 21.54 0.81 (mg Eq AG/g M.S. This variation can be explained as the content of phenolic compounds is influenced by different parameters such as period and location of harvest, climate, geographical conditions, method and extraction time, the solubility and type of solvent used (Naczy and Shahidi 2004).

Our result obtained with 80% methanol extract is 0.30 140 mg Eq Q/g M.S, and this implies that the extract of Globularia alypum have a high level of flavonoids.

Our result is significantly higher than those obtained by (Feriani et al. 2017), which determined the flavonoid content of Globularia methanol extracts as 36.56 3.25 mg Q/g M.S eq, as well as the work of (Khlifi et al.2011) which used methanol as extraction solvent and showed that the flavonoid content in Globularia is 18.20 0.25 mg Eq Q/kg M.S.

Antioxidant activity in vitro

Radical Trapping Activity DPPH: The results of our experiments show that methanol extract has the highest antioxidant activity than other extracts.**Figure 1**

Similar results were founded by (Nour-Eddine et al. 2007).The result of this study suggests that Globularia alypum may be used as a source of antioxidants for pharmacological preparations.

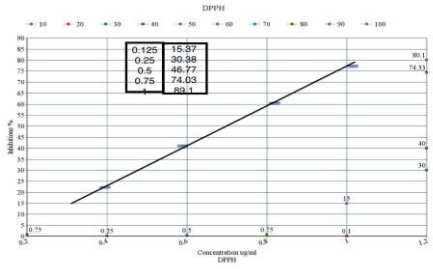

Figure 1: Antiradicalar activity of Globularia methanol extract.

Globularia alypum leaves rich in phenolic compounds are increasingly used in the food industry as they delay oxidative degradation of lipids and improve the quality and nutritional value of foods.

The involvement of polyphenolic compounds in the antioxidant activity of G. alypum is supported by previous results obtained with other species of Globularia (Khlifi et al. 2005).

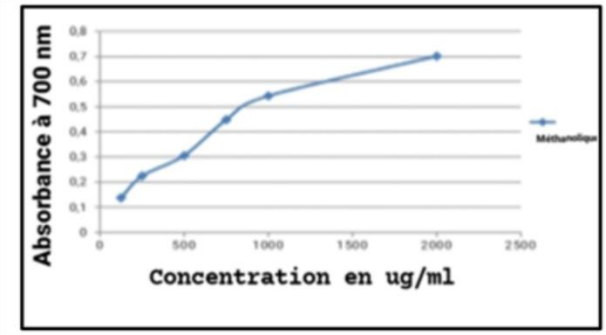

Figure 2: The reducing power of the methanol extract.

It should be noted that the methanol extracts of Globularia alypum were more effective as antioxidant capacity than BHT bleaching test with -Carotene.

Ferric Reducing Antioxidant Power: The evaluation of antioxidant activity by reduction of iron is an easy and reproducible method, for this it is widely used to distinguish the most active extracts (Li et al. 2007). The reductive power of two extracts with deferential is estimated using the (Yildirin et al. 2001).

The results obtained show that the methanolic extract has a reductive power =106,95µg EAA/mg ES at 1mg/ml, The ability of methanolic extract to reduce iron is more important than that of aqueous extract, these results could be due to its high levels of phenolic compounds (polyphenols, flavonoids, tannins...).Figure 2 An important indicator of its antioxidant activity is the ability of electron donation to reflect the reductive power of phenolic compounds and flavonoids (Pokorny et al. 2000).

Through the above results, we can see that the denaturation of BSA by our methanol extract more less than the standard used (sodium diclofenac) for example has a concentration of 800 µg/ml, it reaches an inhibition percentage equal to 85,45% lower than the standard (90.23%) protein denaturation is among the causes of inflammation (Barros et al., 2008) (Bagad et al. 2011). The

production of auto-antigens in inflammatory diseases may be due to denaturation of proteins in vivo. The possible mechanism of denaturation is to inhibit the alteration of electrostatic, hydrogen, hydrophobic and disulfide bonds that maintain the three-dimensional structure of proteins (Barros et al. 2008; Mizushima and Kobayashi., 1968). There is evidence that non-steroidal anti-inflammatory drugs such as phenylbutazone and indomethacin not only inhibit the synthesis of pro-inflammatory prostaglandins, but also inhibit protein denaturation (Sangeetha et al.2011).

CONCLUSION

Medicinal plants still on of the important therapeutic issue for many disease which many drugs faced difficulties and globularia alypum is one of these plants due to its richness of metabolites and active compounds, the power of its antioxidant activity by the methanolic extract open many deep research to explore further for other activity potentials as the search of new therapeutic agent extracted from the plant and its molecular mode of action.

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Conflict of interest: There is no conflict of interest.

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