# Ethnobotanical use, phytochemical study, and antioxidant activity of *Globularia alypum* L.

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## Abstract:

This work aimed to highlight the biological effects of *Globularia alypum* L., a medicinal plant widely used in phytotherapy.

*Globularia alypum* L. is a plant used in traditional medicine in Morocco, the crude extracts prepared from the leaves were obtained using maceration in methanol, ethyl acetate, butanol, and water. The content of total polyphenols was determined using the Folin-Ciocalteu reagent, the first range was for the ethyl acetate extract, followed by butanol, methanol, and aqueous extracts, respectively. The flavonoids amount was determined using the aluminum chloride colorimetric method, the methanolic extract presented the highest content of flavonoids, followed by the one of butanol, then the ethyl acetate and aqueous extracts. The antioxidant activity was carried out using the anti-free radical method based on 2,2-diphenyl-1-picrylhydrazyle (DPPH), the $IC_{50}$ values were estimated for methanolic, ethyl acetate, butanol and aqueous extracts while that of BHT and ascorbic acid. The high phenolic and flavonoid amounts as well as the antioxidant potential of the extracts indicated that the *G. alypum* could be exploited as a source of natural antioxidants.

**Keywords:** Antioxidant activity, DPPH, *Globularia alypum* L., Phytochemical analysis

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## Introduction

Oxidative stress is the result of the disproportion between the production of free reactive species and the capacity of the organism to trap these free radicals by detoxification. This imbalance between the production of free radicals and their detoxification leads to cellular damage that results in pathologies such as diabetes, cancer, and inflammatory diseases. (Favier, 2006) (Bhatti et al., 2022)

Due to the bioactivity of their secondary metabolites, various aromatic and medicinal plants have been described as promising sources of natural antioxidants. Several studies have shown that antioxidant molecules have crucial biological qualities that could be employed in numerous fields such as food preservation, cosmetics, pharmaceuticals, and alternative medicine as natural remedies. (Akbari et al., 2022) The local population widely uses medicinal plants to cure different diseases. Thus, medicinal plants present a promising source to develop new effective drugs.

*Globularia alypum* L. (*G. alypum*) belonging to the Plantaginaceae family is distributed geographically from Europe to North Africa (Mediterranean basin). It is a shrub of 30 to 80 cm, woody and perennial, the floral apparatus with brown-red stems stirred, many leaves and its bushes have blue flowers.

*G. alypum* is well-known for its traditional uses as home remedies, according to an ethnopharmacological survey conducted by Ziyyat et al. in the eastern region of Morocco, *G. alypum* (Ain larnab) leaves are widely used as a laxative agent in the Moroccan. (Ziyyat et al., 1997) The leaves of *G. alypum* are described as a remedy for kidney diseases (Noureddine et al., 2022) in addition to its hypoglycemic, spasmolytic and muscle relaxant effects (Bellakhdar et al., 1991) (Chokri et al., 2010). Recently, Ouffai et al. reported that G. alypum was able to inhibit alpha-amylase and alpha-glucosidase, which are two key enzymes involved in the intestinal digestion of carbohydrates. (Ouffai et al., 2021)

The purpose of the present work was to provide an overview of the ethnobotanical uses of *G. alypum*. in Morocco. to quantify total phenolic flavonoid contents, cytotoxicity, and antioxidant capacity of four extracts of *G. alypum*.

## Materials and Methods:

**Ethnobotanical use of *Globularia alypum* L.** The search started in July until August 2021 based on scientific publication databases, such as Pubmed (https://pubmed.ncbi.nlm.nih.gov/),

web of science (www.webofknowledge.com). These articles were selected when citing the ethnobotanical uses of *G. alypum.*

The search equation is to combine keywords. Such as "*Globularia alypum* AND phytotherapy" OR"*Globularia alypum* AND traditional use" OR *"Globularia alypum* AND ethnobotany +Morocco".

**Plant material**

The plant of *G. alypum* is purchased from an herbalist in the city of Taounate, and the name is then verified by the National Agency of Aromatic and Medicinal Plants in Taounate.

The leaves were washed and cleaned with distilled water, then put to dry at room temperature in an airy place in the shade to better preserve the molecules sensitive to heat and light and reduced to a powder which will constitute the dry matter that will be used for the extraction.

**Chemical reagents and solvents**

L-ascorbic acid, butylated hydroxytoluene (BHT), Folin-Ciocalteu phenol reagent, gallic acid, quercetin, and 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide (MTT), were purchased from Sigma (St. Louis, MO, USA). All other chemicals were of analytical grade.

**Preparation of extracts**

Extraction of the samples was carried out by maceration with methanol, butanol, ethyl acetate and water to obtain four extracts of different polarities. Briefly,50 g of dried leaves of *G. alypum*, were allowed to macerate for 12 hours in 250 ml of each solvent under stirring, and then the macerate was filtered through a Watman 1 filter paper. The extracts obtained were concentrated and removed from the solvent under reduced pressure, using a rotary evaporator (Buchi R-II). At the end of the extraction, All the extracts were preserved in the refrigerator (4°C) until their use for further analysis. (Saidan et al., 2015)

**Determination of total phenolic content**

The total phenolic content of *G. alypum* leaf extracts were determined using the Folin-Ciocalteu colorimetric method, as described by Sanchez-Moreno (Sanchez-Moreno, 2002) with slight modification. Technically, 0.5 mL of the diluted extracts (0.1g/mL), was added to 4 mL of sodium carbonate $Na_2CO_3$ (1M), and 5mL of the Folin-Ciocalteu reagent (10 times diluted in the distilled water), were mixed, the mixture was then thoroughly homogenized and incubated for 30 min at 45 °C. After incubation, the absorbance of each sample was measured at 765 nm by a spectrophotometer. (CECIL1010), against methanol as blank and the total

phenolic content was calculated by using the gallic acid calibration curve (y = 0.0026x + 0.0087, $R^2$ = 0.9978). All assays were performed in triplicate. The Total phenolic content was expressed as mg GAE/g of extract.

**Determination of total flavonoids**

The total flavonoid content of *G. alypum*. Leaves extracts were determined using aluminum chloride colorimetric method previously reported, by Chang et al. (Chang et al., 2020). Briefly, 0.1 mL of each plant extract (1 mg/mL) was mixed with 0.02 mL of aluminum trichloride solution (10 %). 0.02 mL of sodium acetate (1 M), 0.3 mL of methanol and 0.56 mL of distilled water. Then, the mixture was kept standing for 30 min at room temperature. The absorbance was measured against blank by spectrophotometer (CECIL1010) at a wavelength of 415 nm. The total flavonoid content was calculated by using the quercetin calibration curve (y = 0.0074x + 0.1042, $R^2$ = 0.9978). All trials were performed in triplicate. Results were expressed as mg of catechin equivalents per g extracts (mg QE/g).

**DPPH Assay**

The capacity of *G. alypum* extracts to scavenge DPPH radicals, was evaluated according to the method used previously by McDonald et al. (McDonald et al., 2001), and Zouhri et al. (Zouhri et al., 2001). Technically, 50 µL of different dilutions of the extracts were added to 1950 µL of ethanolic solution of DPPH (0.5 mM), The mixture was incubated in the dark for 30 minutes. The absorbance was measured by spectrophotometer (CECIL1010) at 517 nm. Ascorbic acid and BHT were used as positive controls.

DPPH inhibition rates were calculated using the following formula: % of inhibition = [(A control-A sample)/A control] × 100 (A: absorbance). The 50% DPPH inhibitory concentration ($IC_{50}$) was determined from the equation of the percent inhibition versus concentration curve of the different extracts.

**Cytotoxicity**

The 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide (MTT) test was used to analyze cell growth (Amal et al., 2016) (Daoudi et al., 2013). Cells were obtained from sacrificed rabbits. The spleen and thymus were aseptically extracted from the animal and a suspension was prepared by pressing the organ through a fine wire mesh. Cell suspensions were then washed by repeated centrifugation in RPMI medium, and erythrocytes were lysed using 154 mM ammonium chloride. In 96 well plates, 5000 cells were plated per well. After that, the plate was incubated for 72 hours at 37°C in a humidified incubator with 5% CO2.

Before incubation, the prepared extracts were introduced at 0.1 mg/mL to the cells. The optical density (O.D) was measured at 570 nm.

**Statistical analysis**

Results were expressed in triplicates as means± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance, by Graph Pad Prism 8 software. Values of $p<0.05$ were considered statistically significant.

## Results:

**Ethnobotanical use of *G. alypum***

From a geographical standpoint, *G. alypum* is widely reported as a medicinal plant in Chefchaouan, Sefrou, Tata, South of Morocco, and Tafilalet.

*G. alypum* was widely indicated for the treatment of intestinal, genitourinary, respiratory disorders, diabetes, and tuberculosis. This species is used in the case of fever, ulcers, microorganism's infections, laxative, ear infection, inflammation, skin infection, and wounds (Table 1).

Furthermore, according to the data from bibliography the preparation method is linked to the cultural background. For example, the decoction of *G. alypum* leaves is administered in powder form, but it can also use by infusion or maceration for the treatment of diabetes.

The most frequently used parts are leaves and stems, and the used methods of preparation were decoction, infusion, maceration, and cataplasm according to the ethnobotanical reports.

**Total phenolic content**

The phenolic contents were ranged from 183.33 ± 3.25 to 264 ± 1.32 mg GAE/g of extract. Where ethyl acetate extract showed the maximum polyphenols content, in contrast with methanol extract which shown the lowest polyphenols content. The results are shown in the table 2.

Table 1: Ethnobotanical use of *G. alypum* in Morocco

| Area of study | Local man | Ethnobotanical use | Used part | Preparation | administration | reference |
|---|---|---|---|---|---|---|
| Tafilale | Aïn larneb | diabetes | Leaves | n d | n.d | (Eddouks et al., 2002) |
| Imouzzer Ida Outanane | Taslgha | Antiseptic, baldder ailments, burns, diabetes, digestive, skin buttons, | Leaves | Cataplasm, decoction, maceration, powder | oral | (Saadi et al., 2013) |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | tuberculosis, wound healing | | | | |
| Tata Province | Ain larnab – Tasselgha | Hypoglycemic, digestive, laxative, purgative, stimulant, depurative, antiseptic, antimycotic, constipation, gastric ulcers | Leaves | Infusion, decoction | oral | (Abouri et al., 2012) |
| Chefchaouen | Ain larnab | diabetes | Leaves | Infusion | oral | (Merzouki et al., 2002) |
| Central Middle Atlas | Ain larnab – Tasselgha | anti-diabetic | Leaves and Stem with leaves | Infusion, decoction | n.d | (Hachi et al., 2016b) |
| Ksar Lakbir | Ain larnab | Hypoglycemic, digestive, for bilious stimulation and laxative, | leaves | infusion | oral | (Merzouki et al., 2000) |
| Taroudant | Ain larnab – Tasselgha | diabetes | leaves | Decoction | oral | (Katiri et al., 2017) |
| Sidi Slimane | Ain larnab | diabetes | leaves | Decoction | | (Laadim et al., 2017) |
| Sefrou | Taselgha -Âin lerneb | Diabetes mellitus | Flower | Decoction | oral | (Bousta et al., 2014) |
| Fez-Boulemane | Ain larnab | diabetes | leaves | Decoction | oral | (Jouad et al., 2001) |

Table 2. Total polyphenols content from extracts of *G. alypum.*

| Extracts | Total polyphenols (mg EAG /g) |
|---|---|
| Methanol extract | 183.33± 3.25 |
| Butanol extract | 201 ± 1.15 |
| Ethyl acetate extract | 264 ± 1.32 |
| Aqueous extract | 198 ± 2.64 |

Values of total polyphenols content are expressed as concentration mean ± SD (mg EAG /g) of three experiments.

## Total flavonoids content

The flavonoids content of different extracts of *G. alypum* is estimated by the aluminum chloride colorimetric method. the results are represented in the table 3, which shows that the content of flavonoids in the different extracts was 43.04 ± 1.35 mgEQ/g for methanol extract, 41.90 ± 0.83 mg EQ/g for butanol extract, 36.71± 1.67 mg EQ/g for aqueous extract and 30.42 ± 2.17 mg EQ/g for ethyl acetate extract.

Table 3. Flavonoids content from extracts of *G. alypum.*

| Extracts | Flavonoids content (mg EQ /g) |
|---|---|
| Methanol extract | 43.04 ± 1.35 |
| Butanol extract | 41.90 ± 0.83 |
| Ethyl acetate extract | 30.42 ± 2.17 |
| Aqueous extract | 36.71± 1.67 |

Values of flavonoids content are expressed as concentration mean ± SD (mg EQ /g) of three experiments.

## DPPH Assay

Antioxidant properties of different extracts were analyzed using DPPH Assay. The $IC_{50}$ of the DPPH radical, were respectively 93.61± 7.15 µg / ml for Butanolic extract, 90.53± 3.5 µg / ml for the methanolic extract, 83.48± 3.48 µg / ml for the aqueous extract and 97.61± 5.73µg / ml for ethyl acetate extract. (Table 4)

Table 4. Inhibition concentration 50% (IC50, µg/ml) of *G. alypum* by DPPH assay

| Extracts | $IC_{50}$ (µg/mL) |
|---|---|
| Methanol extract | 90.53± 3.5 |
| Butanol extract | 93.61± 7.15 |
| Ethyl acetate extract | 97.61± 5.73 |
| Aqueous extract | 83.48± 3.48 |
| BHT | 174.26 ± 3.67 |
| Ascorbic acid | 34.57 ± 1.84 |

Values of inhibition concentration 50% are expressed as concentration mean ± SD (µg/mL) of three experiments.

Table 5. Phytoconstituents of *G. alypum*

| Study | Part | Extract / Fraction | Active compounds | Molecular formula | Activity |
|---|---|---|---|---|---|
| (Es-Safi et al., 2005) | Aerial Parts | Water-Methanol | 6-hydroxyluteolin 7-O-laminaribioside | $C_{27}H_{30}O_{17}$ | Antioxidant |
| | | | Eriodictyol 7-O-sophoroside | $C_{27}H_{32}O_{16}$ | |
| | | | 6-hydroxyluteolin 7-O-β-d-glucopyranoside | $C_{15}H_{10}O_7$ | |
| | | | luteolin 7-O-sophoroside | $C_{15}H_{10}O_6$ | |
| (Friščić et al.,2022) | Leaf | Methanol | Mannitol | $C_6H_{14}O_6$ | Antioxidant, anti-inflammatory, antimicrobial activities |
| | | | Catalpol | $C_{15}H_{22}O_{10}$ | |
| | | | Aucubin | $C_{15}H_{22}O_9$ | |
| | | | Caffeoylglucoside isomer | $C_{15}H_{18}O_9$ | |
| | | | Verminoside (6-O-Caffeoylcatalpol) | $C_{24}H_{28}O_{13}$ | |
| | | | Geniposide | $C_{17}H_{24}O_{10}$ | |
| | | | Globularioside | $C_{24}H_{29}ClO_{11}$ | |
| (Mohamed et al.,2022) | Leaf | Methanol Aqueous | Gentisic acid | $C_7H_6O_4$ | Attenuate Hyperglycemia |
| | | | Quercetin | $C_{15}H_{10}O_7$ | |
| | | | 4-Hydroxybenzoic acid | $C_7H_6O_3$ | |
| | | | Protocatechuic acid | $C_7H_6O_4$ | |
| | | | Catechin | $C_{15}H_{14}O_6$ | |

| | | | | | |
|---|---|---|---|---|---|
| | | | Apigenin | $C_{15}H_{10}O_5$ | |
| | | | Cinnamic acid | $C_9H_8O_2$ | |
| | | | p-Coumaric acid | $C_9H_8O_3$ | |
| | | | Vanillic acid | $C_8H_8O_4$ | |
| | | | Apigenin 7-O-glucoside | $C_{21}H_{20}O_{10}$ | |
| (Ouffai et al., 2021) | Aerial parts | Diethyl ether fraction | Gallic acid | $C_7H_6O_5$ | antioxidant<br>Alpha-amylase, alpha-glucosidase inhibitory capacity |
| | | | p-Coumaric acid | $C_9H_8O_3$ | |
| | | | Rutin | $C_{27}H_{30}O_{16}$ | |
| | | | Naringenin | $C_{15}H_{12}O_5$ | |
| | | | Quercetin | $C_{15}H_{10}O_7$ | |
| | | Ethyl acetate fraction | Gallic acid | $C_7H_6O_5$ | |
| | | | Rutin | $C_{27}H_{30}O_{16}$ | |
| | | | Ferulic acid | $C_{10}H_{10}O_4$ | |
| | | | Quercetin | $C_{15}H_{10}O_7$ | |

## Cytotoxicity

Cytotoxicity activity of four plant extracts was assessed against splenocytes using the MTT assay. Results obtained in Figure 1 indicate that the four extracts at 0.1 mg/ml showed no cytotoxicity effect against splenocytes.

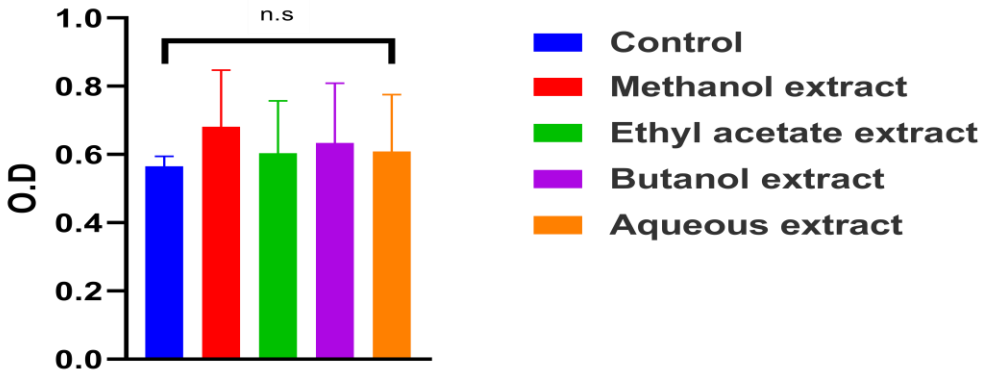


Figure 1: Cytotoxicity of different extracts of *G. alypum*. Values were expressed as mean ± SD of three experiments, Comparison was realized against control (n.s: $p>0.05$)

## Discussion:

The principal objective of this research was to quantify polyphenols, and flavonoids, and to evaluate the antioxidant properties of four extracts of *G. alypum* leaves to prevent cells from oxidative damage.

*G.alypum* is a plant widely used in Moroccan pharmacopoeia (Abouri et al., 2012; Bellakhdar et al., 1991; Bousta et al., 2014; Eddouks et al., 2002; Hachi et al., 2016a, 2016b; Jouad et al.,

2001; Katiri et al., 2017; Laadim et al., 2017; Merzouki et al., 2002, 2000; Saadi et al., 2013; Ziyyat et al., 1997; Ouhaddou et al., 2020) for its therapeutic virtues, according to the results obtained in the bibliographic part. The leaves are the part of the plant most recommended in the management of diabetes and digestive diseases, that's why we have chosen the leaves in our study as the material of study. Moreover, the leaves may contain more bioactive molecules than other parts of the plant and the extraction from leaves is generally easier than from stems therefore the yields can be better.

The total content of the phenolics in the different extracts varied from 183.33± 3.25 to 264 ± 1.32 mg GAE/g of extract. The highest content of polyphenols was obtained in the ethyl acetate extract. followed by butanol extract. then the aqueous extract, and the methanolic extract. For flavonoids content, methanolic extract was in the first range, followed by butanol extract, aqueous extract then the extract of ethyl acetate.

Comparing these results with the available literature on the same species, the values obtained are considerably higher than those reported by Chograni et al (Chograni et al., 2013) who reported that the values of polyphenol and flavonoid content in the extracts of Tunisian *G. alypum* leaves were respectively 22.30 mg GAE /g and 4.72 mg Rutin equivalent per gram of dry weight. Similar considerations can be made when comparing our values with those of (Djeridane et al., 2006) have noticed 4.54 mg QE / g of dry mass of the water ethanol extract (30/70) of the whole plant from *G. alypum*, this result showed that the flavonoids content was five times lower than that of our study. On the other hand (Ouffai et al., 2021) found higher values in the diethyl ether fraction (331.88±17.8 µg GAE/mg total phenolic and 223.46±2.37 µg CE/mg flavonoids). This difference may be due to the difference in part use and solvent of extraction.

The antioxidant activity of the various extracts of *G. alypum*. was determined by the DPPH test, DPPH determines the anti-free radical potency of antioxidants. The antioxidant activity of the aqueous extract was greater than that of all other samples tested, with an $IC_{50}$ of 83.48 ± 3.48 µg / mL, followed by the methanolic extract (90.53± 3.5 µg /mL), butanolic extract (93.61± 7.15 µg / mL), and the extract prepared with ethyl acetate (97.61± 5.73 µg / mL). in agreement with previous studies. (Mohamed et al., 2022) (Ouffai et al., 2021) (Taghzouti, et al., 2016)

It can be concluded that all the extracts exhibited a lower antioxidant activity compared to ascorbic acid (34.57 ± 1.84 µg/mL) and higher than that of BHT (174.26 ± 3.67 µg/mL). The presence of high levels of polyphenols and flavonoids may explain this antioxidant activity.

Indeed, new active compounds were isolated from the hydromethanolic extract of stem and leaves of *G. alypum*, identified as novel phenolic compounds and two known flavonoid glycosides (6-hydroxyluteolin 7-O-β-D glucopyranoside and luteolin 7-O-sophoroside). These compounds have exhibited an interesting antioxidant activity using DPPH test as described by (Es-Safi et al., 2005, 2006, 2007). The antioxidant activity of phenolic compounds is mainly due to their redox properties, which make them act as reducing agents.

In previously published studies on the phytochemistry of the aerial part of *G. alypum*, it was shown that this plant contains numerous secondary metabolites with antioxidant activity, such as polyphenols and flavonoids, which exhibit strong antioxidant activity (see Table 5).

MTT assay was used to determine the cytotoxicity of the four extracts of *G. alypum*, the results showed that the viability of cells did not decrease at 0.1mg / mL of plant extract. in agreement with the results obtained in a previous study. (Hajji et al., 2022)

In another study reporting the chronic toxicity of *G. alypum* in rats over an 8-week period, it was demonstrated that the administration of 0.7 g/kg of G. alypum did not exhibit any toxic effects. (Skim et al., 1998). Even with intraperitoneal administration of different doses of Globularin, a compound isolated from the methanolic extract of *G. alypum*, which showed no mortality up to a dose of 1g/kg body weight in rats. Even at this high dose, (Merghache et al., 2013).

## Conclusion

In this study, we quantified the polyphenol and flavonoid content, as well as the antioxidant activity, in four extracts of G. alypum. The results revealed that the leaves of G. alypum are rich in polyphenols and flavonoids. Although the antioxidant activity of the extracts was lower than that of the positive control, ascorbic acid, it was higher compared to BHT. These findings are promising and suggest that further in vivo studies are needed to explore the potential therapeutic applications of this plant. However, additional in vivo cytotoxicity tests and chemical fractionation are necessary to ensure the safety of this plant and to identify the bioactive compounds.

## References:

Abouri, M., Mousadik, A. El, Msanda, F., Boubaker, H., Saadi, B., & Cherifi, K. (2012). An ethnobotanical survey of medicinal plants used in the Tata Province, Morocco. *International Journal of Medicinal Plant Research*, *1*(7), 99–123. www.internationalscholarsjournals.org

Akbari, B., Baghaei-Yazdi, N., Bahmaie, M., & Mahdavi Abhari, F. (2022). The role of plant-derived natural antioxidants in reduction of oxidative stress. *BioFactors*, *48*(3), 611–633. https://doi.org/10.1002/BIOF.1831

Amal, B., Najlae, M., Ouarda, A., Mohamed, E. K., Alae, C., Abdelali, T., Rachid, B., & Lotfi, A. (2016). Immunomodulating properties of protein fractions isolated from Delphinium staphysagria seeds. *Journal of Medicinal Plants Research*, *10*(3), 29–34. https://doi.org/10.5897/JMPR2015.5957

Bellakhdar, J., Claisse, R., Fleurentin, J., & Younos, C. (1991). Repertory of standard herbal drugs in the Moroccan pharmacopoea. *Journal of Ethnopharmacology*, *35*(2). https://doi.org/10.1016/0378-8741(91)90064-K

Bhatti, J. S., Sehrawat, A., Mishra, J., Sidhu, I. S., Navik, U., Khullar, N., Kumar, S., Bhatti, G. K., & Reddy, P. H. (2022). Oxidative stress in the pathophysiology of type 2 diabetes and related complications: Current therapeutics strategies and future perspectives. *Free Radical Biology and Medicine*, *184*, 114–134. https://doi.org/10.1016/J.FREERADBIOMED.2022.03.019

Bousta, D., Boukhira, S., Aafi, A., Ghanmi, M., & El Mansouri, L. (2014). Ethnopharmacological Study of anti-diabetic medicinal plants used in the Middle-Atlas region of Morocco (Sefrou region) at www.pharmahealthsciences.net. *International Journal of Pharma Research and Health Sciences Available Online*, *2*(1). www.pharmahealthsciences.net

Chang, C.-C., Yang, M.-H., Wen, H.-M., & Chern, J.-C. (2020). Estimation of total flavonoid content in propolis by two complementary colometric methods. *Journal of Food and Drug Analysis*, *10*(3). https://doi.org/10.38212/2224-6614.2748

Chograni, H., Riahi, L., Zaouali, Y., & Boussaid, M. (2013). Polyphenols, flavonoids, antioxidant activity in leaves and flowers of Tunisian Globularia alypum L. (Globulariaceae). *African Journal of Ecology*, *51*(2), 343–347. https://doi.org/10.1111/AJE.12041

Chokri, A., Doukali, R., Abida, K. El, & Cheikh, R. Ben. (2010). Myorelaxant and Spasmolytic Effects of Globularia alypum L. Extract on Rabbit Jejunum. *International Journal of Pharmacology*, *6*(5). https://doi.org/10.3923/ijp.2010.608.615

Daoudi, A., Bousta, D., Aarab, L., & Abdel-Sattar, E. (2013). Evaluation and characterization of the immunomodulatory activity of the protein extract from Citrullus colocynthis L. *Food and Agricultural Immunology*, *24*(1), 47–57. https://doi.org/10.1080/09540105.2011.641168

Djeridane, A., Yousfi, M., Nadjemi, B., Boutassouna, D., Stocker, P., & Vidal, N. (2006). Antioxidant

activity of some algerian medicinal plants extracts containing phenolic compounds. *Food Chemistry*, *97*(4), 654–660. https://doi.org/10.1016/j.foodchem.2005.04.028

Eddouks, M., Maghrani, M., Lemhadri, A., Ouahidi, M. L., & Jouad, H. (2002). Ethnopharmacological survey of medicinal plants used for the treatment of diabetes mellitus, hypertension and cardiac diseases in the south-east region of Morocco (Tafilalet). *Journal of Ethnopharmacology*, *82*(2–3), 97–103. https://doi.org/10.1016/S0378-8741(02)00164-2

Es-Safi, N.-E., Khlifi, S., Kerhoas, L., Kollmann, A., El Abbouyi, A., & Ducrot, P.-H. (2005). Antioxidant Constituents of the Aerial Parts of Globularia a lypum Growing in Morocco. *Journal of Natural Products*, *68*(8), 1293–1296. https://doi.org/10.1021/np0501233

Es-Safi, N.-E., Khlifi, S., Kollmann, A., Kerhoas, L., El Abbouyi, A., & Ducrot, P.-H. (2006). Iridoid Glucosides from the Aerial Parts of Globularia alypum L. (Globulariaceae). *CHEMICAL & PHARMACEUTICAL BULLETIN*, *54*(1). https://doi.org/10.1248/cpb.54.85

Es-Safi, N.-E., Kollmann, A., Khlifi, S., & Ducrot, P.-H. (2007). Antioxidative effect of compounds isolated from Globularia alypum L. structure–activity relationship. *LWT - Food Science and Technology*, *40*(7), 1246–1252. https://doi.org/10.1016/j.lwt.2006.08.019

Favier, A. (2006). Stress oxydant et pathologies humaines. *Annales Pharmaceutiques Françaises*, *64*(6), 390–396. https://doi.org/10.1016/S0003-4509(06)75334-2

Friščić M, Petlevski R, Kosalec I, Madunić J, Matulić M, Bucar F, Hazler Pilepić K, Maleš Ž. *Globularia alypum* L. and Related Species: LC-MS Profiles and Antidiabetic, Antioxidant, Anti-Inflammatory, Antibacterial and Anticancer Potential. *Pharmaceuticals*. 2022; 15(5):506. https://doi.org/10.3390/ph15050506

Hachi, M., Ouafae, B., Hachi, T., Mohamed, E. B., Imane, B., Atmane, R., & Zidane, L. (2016a). Contribution to the ethnobotanical study of antidiabetic medicinal plants of the Central Middle Atlas region (Morocco). *Lazaroa*, *37*, 135–144. https://doi.org/10.5209/LAZAROA.51854

Hachi, M., Ouafae, B., Hachi, T., Mohamed, E. B., Imane, B., Atmane, R., & Zidane, L. (2016b). Contribution to the ethnobotanical study of antidiabetic medicinal plants of the Central Middle Atlas region (Morocco). *Lazaroa*, *37*, 135–144. https://doi.org/10.5209/LAZAROA.51854

Hajji, N., Bayar, S., Zouari, N., Altayb, H., Sebai, H., & Chaieb, K. (2022). Molecular Docking Studies, Chemical Composition, Antioxidant, Cytotoxicity, Antibacterial and Antifungal Activities of Globularia alypum Extract. *Current Bioactive Compounds*, *18*(3). https://doi.org/10.2174/1573407217666210831160746

Jouad, H., Haloui, M., Rhiouani, H., El Hilaly, J., & Eddouks, M. (2001). Ethnobotanical survey of medicinal plants used for the treatment of diabetes, cardiac and renal diseases in the North centre region of Morocco (Fez-Boulemane). *Journal of Ethnopharmacology*, *77*(2–3), 175–182. https://doi.org/10.1016/S0378-8741(01)00289-6

Katiri, A., Barkaoui, M., Msanda, F., & Boubaker, H. (2017). Ethnobotanical Survey of Medicinal Plants Used for the Treatment of Diabetes in the Tizi n' Test Region (Taroudant Province, Morocco). *J Pharmacogn Nat Prod*, *3*, 1. https://doi.org/10.4172/2472-0992.1000130

Laadim, M., Ouahidi, M. L., Zidane, L., Hessni, A. El, Ouichou, A., & Mesfioui, A. (2017). *Journal of Pharmacognosy and Phytotherapy Ethnopharmacological survey of plants used for the treatment of diabetes in the town of Sidi Slimane (Morocco)*. *9*(6), 101–110. https://doi.org/10.5897/JPP2016.0437

McDonald, S., Prenzler, P. D., Antolovich, M., & Robards, K. (2001). Phenolic content and antioxidant activity of olive extracts. *Food Chemistry*, *73*(1), 73–84. https://doi.org/10.1016/S0308-8146(00)00288-0

Merghache, S., Zerriouh, M., Merghache, D., Tabti, B., Djaziri, R., & Ghalem, S. (2013). Evaluation of hypoglycaemic and hypolipidemic activities of Globularin isolated from Globularia alypum L. In normal and streptozotocin-induced diabetic rats. *Journal of Applied Pharmaceutical Science*, *3*(4), 1–7. https://doi.org/10.7324/JAPS.2013.3401

Merzouki, A., Ed-derfoufi, F., & Molero Mesa, J. (2000). Contribution to the knowledge of Rifian traditional medicine. II: Folk medicine in Ksar Lakbir district (NW Morocco). *Fitoterapia*, *71*(3), 278–307. https://doi.org/10.1016/S0367-326X(00)00139-8

Merzouki, A., F Ed-derfoufi, & J Molero-mesa. (2002). Contribución al conocimiento de la medicina rifeña tradicional III: Fitoterapia de la diabetes en la provincia de Chefchaouen (norte de Marruecos) | Ars Pharmaceutica (Internet). *Ars Pharmaceutica*, *44*(1), 59–67. https://revistaseug.ugr.es/index.php/ars/article/view/5125

Mohamed, T., Souiy, Z., Achour, L., & Hamden, K. (2022). Anti-obesity, anti-hyperglycaemic, anti-antipyretic and analgesic activities of Globularia alypum extracts. *Archives of Physiology and Biochemistry*, *128*(6), 1453–1460. https://doi.org/10.1080/13813455.2020.1773865

Mohamed, T., & Hamden, K. (2022). Globularia alypum Extracts Attenuate Hyperglycemia and Protect against Various Organ Toxicities in Alloxan-Induced Experimental Diabetic Rats. Evidence-Based Complementary and Alternative Medicine, 2022.

Noureddine, B., Mostafa, E., & Mandal, S. C. (2022). Ethnobotanical, pharmacological, phytochemical, and clinical investigations on Moroccan medicinal plants traditionally used for the management of renal dysfunctions. *Journal of Ethnopharmacology*, *292*, 115178. https://doi.org/10.1016/J.JEP.2022.115178

Ouffai, K., Azzi, R., Abbou, F., Mahdi, S., El Haci, I. A., Belyagoubi-Benhammou, N., Bekkara, F. A., & Lahfa, F. B. (2021). Phenolics compounds, evaluation of Alpha-amylase, alpha-glucosidase inhibitory capacity and antioxidant effect from Globularia alypum L. *Vegetos*, *34*(2), 477–484. https://doi.org/10.1007/s42535-021-00211-3

Ouhaddou, H., ALAOUI, A., & Sezgin, AYAN (2020). Ethnobotanical survey of medicinal plants used for treating diabetes in Agadir Ida Outanane region, Southwestern Morocco. Arabian Journal of Medicinal and Aromatic Plants, 6(2), 72-86.

Saadi, B., Msanda, F., & Boubaker, H. (2013). Contributions of folk medicine knowledge in South-western Morocco: The case of rural communities of Imouzzer Ida Outanane Region. *International Journal of Medicinal Plant Research*, *2*(1), 135–145. www.internationalscholarsjournals.org

Saidan, N. H., Shahrul, M., Hamil, R., Memon, A. H., Abdelbari, M. M., Hamdan, M. R., Mohd, K. S., Shah, A. M., Majid, A., & Ismail, Z. (2015). Selected metabolites profiling of Orthosiphon stamineus Benth leaves extracts combined with chemometrics analysis and correlation with biological activities. *BMC Complementary and Alternative Medicine*. https://doi.org/10.1186/s12906-015-0884-0

Sanchez-Moreno, C. (2002). Review: Methods Used to Evaluate the Free Radical Scavenging Activity in Foods and Biological Systems. *Food Science and Technology International*, *8*(3). https://doi.org/10.1106/108201302026770

Skim, F., Lazrek, B. H., El Amri, H., Kaaya, A., & Jana, M. (1998). Toxicological studies on Globularia alypum and Zygophyllum gaetulum in rats. *Phytotherapy Research*, *12*(8), 592–594.https://doi.org/10.1002/(SICI)1099-1573(199812)12:8<592::AID-PTR348>3.0.CO;2-Z

Taghzouti, O. K., Balouirib, M., Ouedrhiric, W., Chahadd, A. E., & Romanea, A. (2016). In vitro evaluation of the antioxidant and antimicrobial effects of Globularia alypum L. extracts. J. Mater. Environ. Sci, 7, 1988-1995.

Ziyyat, A., Legssyer, A., Mekhfi, H., Dassouli, A., Serhrouchni, M., & Benjelloun, W. (1997). Phytotherapy of hypertension and diabetes in oriental Morocco. *Journal of Ethnopharmacology*, *58*(1). https://doi.org/10.1016/S0378-8741(97)00077-9

Zouhri, A., Bouddine, T., El-mernissi, Y., El-Akhal, J., Hajji, L., & Amhamdi, H. (2022). Syzygium aromaticum: Traditional uses, antioxidant, anti-inflammatory activities, and photo-protective properties. Arabian Journal of Medicinal and Aromatic Plants, 8(3), 43-56.