INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 9, No. 6, p. 460-467, 2016

RESEARCH PAPER OPEN ACCESS

Ethnobotany study and phytochemical screening of *Caralluma europaea* (Guss.) N.E. Br.

Nadia Sagou*[1], Zineb Rhandour[1], Malak Eddebbagh[1], Mohamed Hsaine[2], Mohamed Berrada[1], Abdelmjid Abourriche[1], Ahmed Bennamara[1]

[1]*Laboratory of Biomolecules and Organic Synthesis, Faculty of Sciences Ben M'sick, University Hassan II Casablanca, Morocco*

[2]*Laboratory of Ecology and Environment/Department of Biology, Faculty of Science Ben M'sik, University Hassan II Casablanca, Morocco*

Key words: *Caralluma europaea*, Ethnobotanical study, Phytochemical screening

http://dx.doi.org/10.12692/ijb/9.6.460-467 Article published on December 31, 2016

Abstract

Natural substances derived from plants have multiple interests used in different industries. Moreover, the use of synthetic molecules is currently being called into question, because of the potential toxicological risks of these molecules. Now, new natural plant sources are being sought. The study of the pharmacological activities of plants has become a kind of fashion in the last decade, because of the socio-economic and cultural changes of consumers and industrialists over time; this has upset the scientific community and prompted it to investigate in this search axis. The ethno botanical information obtained from the traditional therapists of the big souk of the city of Marrakech and that of the city of Casablanca enabled us to note that the use of raw aerial parts in dried juice or powder mixed with honey from *Caralluma europaea* (Guss.), A medicinal plant belonging to the Apocynaceae family, locally known as "Daghmouss", is highly appreciated by the local population for its therapeutic properties. *Caralluma europaea* (Guss.) is a spontaneous species, very widespread in mountainous regions of Morocco, contributes significantly to health care for the treatment of some diseases such as genital cysts, diabetes, goitres and kidney stones. Various phytochemical tests applied to the aqueous and organic extracts of the stems of *Caralluma europaea* (Guss.) have shown the presence of a few families of secondary chemical compounds, in particular polyphenols, flavonoids, tannins and alkaloids, known bioactive compounds for their phytochemical action Antibacterial and antifungal effects to anti-cancer and anti-tumor effects and because of which *Caralluma europaea* (Guss.) Has significant therapeutic potential that we plan to expand.

* **Corresponding Author:** Nadia Sagou ✉ nadiasag@hotmail.com

Introduction

Plants have always played a vital role in medical care, and phytotherapeutic means are rich, and continue to be enriched with new natural substances. Therefore, it is important to make a scientific evaluation of the medicinal plants of our country which constitute a very important natural wealth in Morocco (Bellakhdar J., 1997; Ait Oukrouch I., 2015), whose valorisation makes it possible to obtain a maximum of information relating to their toxicological, physiological and pharmacodynamic effectiveness; correlated by the active ingredients they contain.

These properties depend on the presence of various bioactive agents and belonging to different chemical classes. Such studies would lead to the development of drugs that can be used in primary health care at lower cost. Therefore, recent work aims to isolate new substances from plants and to find other ways of application in different fields.

This study focuses on a species known in Morocco for its therapeutic benefits, which is known as the *Caralluma europaea* (Guss.) NE Br, one of the species of the Apocynaceae family and belonging to the genus *Caralluma* which is represented by seven species in Morocco (Meve U., Heneidak S., 2005; Audissou J.A., 2005). Its vernacular name is "Daghmouss" in Arabic and Fingers of God in French (Benkhnigue O., 2014). *Caralluma europaea* (Guss.) is distributed from Morocco and southern Spain along the North African coast from the Mediterranean to Sinai (Bensusan K., 2009). It is the only representative to grow freely on the European continent (Jonkers B and Walker CC., 1993). It is also found on the island of Lampedusa (Italy) where it was discovered first by Gussone in 1832. In Morocco, it is found in the interior of the country, in the Anti-Atlas, the Middle Atlas and the Rif. It is a fat plant, of cacti form aspect, with stems more or less succulent (Fig. 1), fleshy and with rudimentary leaves. The pentamerous flower with corolla of 10-12 mm rotaced, fleshy, brownish more or less spotted, with triangular lobes very obtuse more or less ciliated on the edges (Fig. 2). The fruit is formed of two dehiscent follicles containing small seeds (Aafi A *et al*,, 2002).

To our knowledge the traditional use of *Caralluma europaea* (Guss.) is limited to very restricted medicinal purposes and no work relating to the phytochemistry of this species exists in the literature except two works, one on essential oils (Zito *et al*., 2010) and the other on volatile products (Formisano C *et al*., 2009). In order to fill this gap, we have undertaken the present study on ethnobotany and the phytochemical screening of *Caralluma europaea* (Guss.).

The objective of our work is to carry out an ethno botanical study and a phytochemical screening in order to bring out the causal relationship between the usual and traditional use coupled with the ethno botanical specifications of this plant and its phytochemical potential.

Fig. 1. Stems and flower of *Caralluma europaea*

Fig. 2. Single Flower of *Caralluma europaea*

Materiels and methods

The method of work consisted of proceeding ethno botanical investigations. They took place in three stages in the period of May-June 2016; firstly the direct interview on the use of *Caralluma europaea* (Guss.) and the recommended recipes for treatment with ten traditional therapists and a population of 20 users of traditional medicine, 50% female and 50% male; Secondly the field trip in search of the plant in question and finally the identification of the different crops.

Plant material

It consists of stems of *Caralluma europaea* harvested at the mountain Ighri 5 km from the town of Demnat (Province of Tadla-Azilal) on the way to Iminifri (Fig. 3), in May and June 2016. A specimen of the species has been identified by Professor M. Hsaine Department of Biology/laboratory of Ecology and Environnement of the Faculty of Sciences Ben Msik Casablanca, Morocco.

Fig. 3. Town of Demnat and around satellite view.

Extraction methods

Various types of extracts were prepared from the dried powder of the aerial part of *Caralluma europaea* (Guss.). In order to preserve the wholeness of the molecules and to minimize any fermentation which could degrade the organic matter, the aerial part was dried at low temperature, protected from light, in a ventilated place and then crushed.

Long-term maceration assisted extraction

Maceration in various solvents of increasing polarity was carried out as follows, 5g of dried powder were contacted with 100 ml of ethyl acetate and stirred for 72 h at room temperature and protected from the light.

After centrifuging and filtration, the remaining residue was then stirred with the five solvents (hexane, dichloromethane, methanol, ethanol and distilled water) each separately in accordance with the technique used above. All the resulting extracts were concentrated under vacuum at 50°C in the rotavapor (Diallo and *et al.,* 2004).

Ultrasonic assisted extraction

They are carried out by successive exhausting of the plant powder using the same solvents used for extraction by long-term maceration. The procedure was as follows: 2.5 g of powder needs to be put in contact with 50 ml of each solvent separately in an ultrasonic bath at room temperature and protected from light for one hour (Vinatoru M. *et al.*, 1997).

The mixture is then centrifuged and filtered. All the resulting extracts were concentrated in vacuum at 50°C in a rotavapor

Extraction yield

The extraction yield is calculated by the formula given by Falleh *et al* (Falleh H.; Ksouri R.; Chaieb K.; Karray-Bouraoui N., Trabelsi N.; Boulaaba M. and Abdelly C., 2008):

R (%) = 100 x (M. ext / M.ech)

Where R is the % yield; M. ext is the mass of the extract after evaporation of the solvent in mg and M. ech is the mass of the dried plant sample in mg.

Phytochemical screening

The different groups of compounds (sterols, polyterpenes, polyphenols, flavonoids, tannins, alkaloids and saponins) contained in the extracts were demonstrated by a study based on solubility tests, staining and precipitation reactions according to the methods described by (Ronchetti F. & Russo G., 1971), (Hegnauer R., 1973), (Wagner H., 1983), (Békro Y. A. *et al,*, 2007).

Sterols and polyterpenes

To demonstrate the sterols and the polyterpenes, we used the LIEBERMANN reagent. Indeed, 5 ml of each of the twelve extracts of the plant was evaporated to dryness in a capsule on a water bath. The residue is dissolved hot in 1 ml of acetic anhydride. Then, 0.5ml of concentrated sulfuric acid was added to the triturate. The appearance, at the interphase, of a purple and purple ring, turning blue and green, indicates a positive reaction. This test was performed with a chloroform control solution of cholesterol.

Polyphenols

To demonstrate the polyphenols, the reaction with ferric chloride ($FeCl_3$) was used. Thus, to 2ml of each liquid extract is added a drop of alcoholic solution of 2% ferric chloride. Ferric chloride causes, in the presence of polyphenolic derivatives, the appearance of a darker blue or greenish-blue coloration. The control is carried out with the alcoholic solution of gallic acid.

Flavonoids

To demonstrate the flavonoids, the so-called cyanidin reaction was used. 2ml of each extract were evaporated and the residue was taken up in 5ml of hydrochloric alcohol diluted 2 times. By adding 2 to 3 chips of magnesium, there is a release of heat and then a pink-orange or purplish coloration. The addition of 3 drops of is amyl alcohol intensified this coloring which confirmed the presence of flavonoids. An alcoholic solution of quercetin was used as a control.

The tannins

● The catechic tannins are identified by the STIASNY reagent (Formal 30%, concentrated HCl: 1/0.5). 5ml of each extract was evaporated to dryness. After adding 15ml of the STIASNY reagent to the residue, the mixture was held in a water bath at 80°C for 30min. The observation of a precipitate in large flakes characterizes the catechictanins.

● Gallic tannins are identified by addition of $FeCl_3$. Indeed, we filtered the previous solution. The filtrate is collected and saturated with sodium acetate. The addition of 3 drops of $FeCl_3$ at 2% gives rise to an intense blue-black coloring indicating the presence of gallic tannins. An alcoholic solution of gallic acid is used as a control.

The alkaloids

To demonstrate the alkaloids, the reagents of DRAGENDORFF (iodobismuthate reagent) and BOUCHARDAT (iodoiodidereagent) were used. Indeed, 6 mL of each solution were evaporated to dryness. The residue is taken up in 6 ml of alcohol at 60°C. The addition of 2 drops of the Dragendorff reagent to the alcoholic solution causes a precipitate or an orange coloration. The addition of 2 drops of the Bouchardat reagent to the alcoholic solution caused a precipitate of reddish brown color and indicated a positive reaction.

Saponins

To demonstrate the saponins, we introduced 10 mL of each of the aqueous extracts into a test tube. The tube is stirred for 15 seconds then allowed to stand for 15 min. A height of persistent foam, greater than 1 cm indicates the presence of the saponosides.

Results and discussion

Ethno botany Study

The interview we had with the ten traditional therapists of the big souk of the city of Marrakech and that of the city of Casablanca as well as the population consists of a set of questions focused on the vernacular name of the plant, the use geographical distribution and method of preparation. The results of this interview revealed the following points:

- Both the traditional therapists and the users of this plant agree on the vernacular name "Daghmouss"
- Traditional therapists confirm that the plant is perennial, harvested almost all year in the mountainous regions (Demnat, Azilal,) but in cold weather the harvest decreases due to the difficulties of accessing it
- *Caralluma europaea* is used predominantly by the female sex, because it is known to have virtues for the treatment of cysts of the female reproductive system
- Both men and women use *Caralluma* stems in decoction or powder for the treatment of multiple diseases ranging from simple (cough, cold) to very complicated (diabetes, goitres, kidney stones, genital cysts)
- Traditional therapists recommend that consumers use fresh or dried *Caralluma* stems mixed with milk or mixed with honey.

Indeed the treatment of these diseases by *Caralluma* belongs to the ancestral heritage of the Traditional therapy of the soil used in the mountainous regions where this plant grows.

Yield

The calculations of all the yields of the extractions carried out are grouped in Table 1. Regardless of the extraction technique, when comparing solvents, water, methanol, ethanol and dichloromethane, they are much more effective (efficiency greater than 7%) than hexane and ethyl acetat (yield of less than 7%).

In the case of the long-term maceration assisted extraction, good yields (from 9.12% to 30.34%) were obtained whatever the solvent. On the other hand, the hexane does not make it possible to obtain sufficient yields with the two extraction techniques. The higher efficiency of polar and moderately polar solvents can be explained, on the one hand, by their polarities, hence an efficient penetration and, on the other hand, due to their extracting power. This promotes the penetration and diffusion of the solvent into the plant material containing the bioactive compounds and thus facilitates the release of the latter.

Table 1. Extraction yield.

Types of extraction	Solvents	Mass of extract	Yield	Color
Extraction by 72 hours maceration	Ethyl acetate	0,320g	6,4%	Light green
	Hexane	0,143g	2,86%	Light green
	dichloromethane	0,636g	12,72%	Very dark green
	Methanol	0,791g	15,82%	dark green
	Ethanol	0,458g	9,12%	dark green
	Distilled water	1,517g	30,34%	orange
Ultrasonic assisted extraction	Ethyl acetate	0,110g	4,4%	Light green
	Hexane	0,042g	1,68%	Light green
	dichloromethane	0,175g	7%	Very dark green
	Methanol	0,299g	11,96%	dark green
	Ethanol / Distilled water	0,340g	13,6%	dark green
	Distilled water	0,518g	20,72%	orange

Distilled water is the solvent which produced the largest amount of extracted mass (30.34%) for maceration extraction and (20.72%) for assisted extraction Ultrasound, and hexane.

The smallest extracted mass (2.86%) for maceration extraction and (1.68%) for ultrasound assisted extraction. It was found that the greatest yield was obtained by long-term maceration assisted extraction.

Phytochemical screening

The phytochemical screening of *Caralluma europaea* (Guss.) stems was carried out for the first time and the results are summarized in Table 2. These results revealed that:

● Sterols and polyterpenes are present in all extracts with maximum abundance for the ethanoic extract by long-term maceration

● Dichloromethane, ethanoic and methanoic extracts showed the maximum abundance for polyphenols except for extraction with dichloromethane assisted by ultrasound, the test was moderately positive. This can be explained by the fact that there are polyphenols of lipophilic character extracted by moderately apolar solvents and polyphenols of a more polar nature extracted by alcohols;

● Flavonoids are poorly present in the extracts by apolar solvents (ethyl acetate, hexane and dichloromethane), while the methanoic and aqueous ethanoic extracts show a moderately positive test;

● The same for tannins, they are weakly present in the extracts by apolar solvents and moderately present in the extracts by polar solvents;

● For alkaloids, the test is strongly positive for the ethanoic and methanoic extracts as well as for the ethyl acetate extract;

● The saponins test for both types of extraction is moderately positive.

Table 2. Results of phytochemical screening.

extracts	SterolsAnd Poly-terpenes	Poly-phenols	Flavonoids	Tannins	Alkaloids	Saponins
AcEt(M)	++	++	+	+	+++	/
HEX(M)	++	++	+	+	++	/
DIC(M)	++	+++	+	+	++	/
MEOH (M)	++	+++	++	++	+++	/
ETOH(M)	+++	+++	++	++	+++	/
ED(M)	+	++	++	++	++	+++
AcEt(U)	++	++	+	+	+++	/
HEX(U)	++	++	+	+	+	/
DIC(U)	++	++	+	+	+++	/
MEOH(U)	+	+++	++	++	+	/
ETOH (U)	+	+++	++	++	++	/
ED(U)	-	++	++	++	++	+++

Ac Et: ethyl acetate; HEX: hexane; DIC: dichloromethane; MEOH: methanol; ETOH: ethanol;
ED: distilled water

- : Negative test; + : Low positive test; ++: Positive test; +++: Highly positive test ; / : Not tested.
M: maceration; U: ultrasonic.

It is clear from the results that the plant material can contain variable amounts of phytochemicals and that this diversity is affected firstly by the polarity of the solvents used, that is to say that the polar solvents (water, ethanol And methanol) showed a maximum number of phytochemicals except for a few compounds, whereas hexane and ethyl acetate showed only a moderate or

low and secondly by the chemical nature of these secondary compounds in the plant which varies from simple to highly polymerized compounds.

Conclusion

Despite the medicinal importance of *Caralluma europaea* (Guss.), this species has previously been studied from a biological point of view and only two works from a chemical point of view (Zito and Forminaso). However, the present study has demonstrated the great richness of the plant in polyphenols, flavonoids, tannins, alkaloids and saponins, natural products of considerable interest in the pharmacological field. This work therefore provides a phytochemical contribution to the knowledge of *Caralluma europaea* (Guss.) and thus allows us to better understand the therapeutic properties of the extracts of this plant. It would therefore be very interesting to exploit these extracts for the research of their active ingredients, which are responsible for their pharmacological properties.

References

Aafi AM, Taleb-Fechtal M. 2002. Espèces remarquables de la flore du Maroc Centre National de Recherche Forestière.

Ait Oukrouch I. 2015. Enquête ethnobotanique à propos des plantes médicinales utilisées dans le traitement traditionnel du diabète de type II à Marrakech. Thèse de Doctorat en Médecine.

Audissou JA. 2005. Moroccan Stapeliads. Asklepios **94,** 18-27.

Békro YA, Békro JAM, Boua BB, Tra BFH, Ehilé EE. 2007. Etude ethnobotanique et screening phytochimique de *Caesalpinia benthamiana* (Baill.) (Caesalpiniaceae). Rev. Sciences & Nature Vol. 4 N° **2,** 217-225.

Bellakhdar J. 1997. La pharmacopée marocaine traditionnelle. Médecine arabe ancienne et savoirs populaires. Edition Le Fennec, Paris, Ibis press 759-764 p.

Benkhnigue O, Ben Akka F, Salhi S, Fadli M, Douira A, et Zidane L. 2014 Catalogue des plantes médicinales utilisées dans le traitement du diabète dans la région d'Al Haouz-Rhamna (Maroc). Journal of Animal & Plant Sciences, Vol. 23, Issue **1,** 3545-3549.

Bensusan K. 2009.-Taxonomy and conservation status of Moroccan Stapeliads (Apocynaceae – Asclepiadoideae - Ceropegieae - Stapeliinae) Bulletin de l'Institut Scientifique, Rabat, section Sciences de la Vie, n° **31(2),** 67-77.

Diallo, Sanogo R, Yasambou H, Traoré A, Coulibaly K, Maiza A. 2004. Etude des constituants des feuilles de *Ziziphus mauritiana* Lam. (Rhamnaceae) utilisées traditionnellement dans le traitement du diabète au Mali. C. R. Chimie.

Falleh H, Ksouri R, Chaieb K, Karray-Bouraoui N, Trabelsi N, Boulaaba M, Abdelly C. 2008. Phenolic composition of *Cynara cardunculus* L, organs and their biological activitie. Compt. Rend. Biol. Vol. **331.**

Formisano C, Senatore F, Della Porta G, Scognamiglio M, Bruno M, Maggio A, Rosselli S, Zito P, Sajeva M, 2009 Headspace volatile composition of the flowers of *Caralluma europaea* N.E. Br. (Apocynaceae). Molecules **14,** 4597-4613.

Hegnauer R. 1973. Chemotaxonomie der Pflanzen, Bikhäuser Verlag, Basel, Suttgart **6,** 761 pp.

Jonkers B, Walker CC. 1993. The asclepiads of Morocco–a short commentary. Asklepios **59,** 14-21.

Meve U, Heneidak S. 2005. A morphological, karyological and chemical study of the *Apteranthes* (*Caralluma*) *europaea* complex. Botanical Journal of the Linnean Society **149,** 419-432.

Ronchetti F. Russo G. 1971. A new alkaloid from *Rauvolfia vomitoria*. Phytochemistry, Vol. **10,** 1385-1388.

Vinatoru M, Toma M, Radu O, Filip PI. 1997. The use of ultrasound for the extraction of bioactive principles from plant materials. Ultrasonics Sonochemistry.

Wagner H. 1983. Drogenanalyse, Dünschichtc hromatographische Analyse von Arzneidrogen. Springer Verlag Berlin Heidelberg New York 522 pp.

Zitoand, 2010. Essential oil composition of steams and fruits of *Caralluma europaea* N.E. Br; Molecules **15,** 627-638.