Spectral characterization and colorimetric determination of extracts of *Caralluma europaea* (Guss.)

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ABSTRACT

The present study focuses on spectral analysis by two methods, Fourier Transform Infrared Spectroscopy and Mass Spectrometry Gas Chromatography of aqueous and organic solvent extracts of increasing polarity (hexane, dichloromethane, ethyl acetate, ethanol and methanol) from the aerial part of *Caralluma europaea* (Guss.), as well as the colorimetric determination of polyphenols, flavonoids and tannins present in these extracts. The FT-IR spectrum showed the presence of different groups which may originate from the phytochemicals. GC-MS analysis revealed the presence of several compounds, mainly saturated and unsaturated fatty acids, hydrocarbons, glycerin, tocopherol and squalene. The best determinations of polyphenols and flavonoids of the dry matter of *Caralluma europaea* (Guss.) are obtained by maceration, although generally long, it seems the best method of extracting these fragile molecules. On the other hand, ultrasound assisted extraction is more effective in obtaining the best dosage of condensed tannins. These results confirm that this plant is rich in important bioactive constituents, so further scientific research is needed.

Key words:*Caralluma europaea* (Guss.), FT-IR analysis, GC-MS analysis and colorimetric

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INTRODUCTION

Plants produce bioactive compounds to protect themselves, but several studies have shown that these plants can also protect humans against infectious diseases [1-2]. Thus, there is great interest in the development of plants of medicinal interest as sources of natural bioactive substances [3-4]. However, each plant species can contain up to thousands of different constituents, which are used in the food, cosmetic and pharmaceutical industries [5-8]. Among these compounds, the polyphenols which constitute a family of compounds which are omnipresent in the plant kingdom [9-10]. Many properties of these health-related compounds, widely described in the bibliography, are mainly based on their biological activities [11].

Caralluma europaea (Guss.) Is an attractive and succulent medicinal plant of the family Apocynaceae which comprises about 200 genera and 2500 species [12] with extensive distribution in Egypt, Spain, Italy (Lampedusa Island), Libya, In Tunisia, Algeria and Morocco [13-14], and represented in all types of habitats. Seven species of Caralluma is found in Morocco [15]. It has quadrangular stems up to 15-20 cm in diameter and forms large tufts, the flowers are red-brown with yellow stripes with a diameter of 10-15 mm [16]

The presence of pregnane steroids in several species of Caralluma has been reported in earlier chemical studies [17-20] and it may indicate systematic importance in the genus. To our knowledge there are few studies on the chemical composition of *Caralluma europaea* (Guss.) and its traditional use is restricted to very restricted medicinal purposes and no work related to spectral analysis as well as the polyphenol dosage of this No species existed in the literature. There are four published works: the first is Meve and Heneidak [21], the second is Zito et al, [22] the essential oils, and the third one deals with volatile products made by Formisano et al , [23] and the last one concerning the ethnobotanical study and the phytochemical screening Sagou. N et al., [24].

In order to fill this gap, we have undertaken the present study to determine the functional groups by spectral analysis (FT-IR), the search for bioactive compounds by mass spectrometric analysis as well as the determination of total phenols, flavonoids and condensed tannins present in the aqueous and organic extracts of the solvents of increasing polarity (hexane, dichloromethane, ethyl acetate, ethanol and methanol) obtained from the aerial part of Caralluma europaea (Guss.).

MATERIALS AND METHODS

Plant material

The aerial part of *Caralluma europaea* (Guss.) was collected at the mountains of the city of Demnat, Tadla-Azilal Province, Morocco. The specimens were examined carefully and in order to preserve the integrity of the molecules and to minimize any fermentation which might degrade the organic matter, the aerial parts were dried at low temperature, protected from light, in a place Aerated and then crushed. Different types of extracts were prepared from the dried powder of the aerial parts of *Caralluma europaea* [24] and the extraction was carried out by two methods, assisted extraction by long-term maceration and ultrasound-assisted extraction.

Analysis by Fourier Transform Infrared Spectroscopy (FT-IR)

One milligram of each extract was mixed with 100 mg of KBr (FT-IR grade) and then compressed to prepare a salt disc with a diameter of 3 mm. The disc was immediately stored in the sample holder and the FT-IR spectra were recorded in the absorption range between 400 and 4000 cm -1. All research was carried out using a Bruker FT-IR spectrometer.

Analysis by gas chromatography coupled to mass spectrometry (GC-MS)

1 milligram of each extract was mixed with two milliliters of ethanol, stirred vigorously and filtered on filter paper, followed by injection of 1 µl into the GC injector coupled to SHIMADZU mass spectrometry. Separation of the chemical compounds present in the extracts was carried out by gas chromatography using a capillary column having the thickness

of 0.25 µm, helium serving as carrier gas with a pressure of 70 kPa, the temperature of the furnace being Started at 60 °C and then increased to 120 °C at a rate of 15 °C/min and then increased to 260 °C at a rate of 20 °C/min. This last temperature is maintained for 25 minutes. Mass spectra were obtained by mass spectrometric analysis with the impact of electrons (EI) at 70eV of electron energy and scanning from 50 to 500 m/z. All the spectra obtained were screened by the NIST database 2011.

Determination of Total Phenol Content

Determination of the total phenol content was carried out according to the method of Singleton and Rossi [25] using the Folin-ciocalteu reagent. 0.4 ml of each diluted extract is added 2 ml of Ciocalteu folin (diluted 10-fold), after standing for 5 minutes, 1.6 ml of sodium carbonate Na_2 CO_3 (75 g / L) is added. The whole is incubated for two hours in the absence of light and at ambient temperature. The absorbance was measured at 760 nm by a UV-3100PC VWR Spectrophotometer. The contents are calculated according to the following equation: A = 0.0036 C + 0.0183 with R2 = 0.998 (Fig 1). This equation is obtained from the calibration curve established with different concentrations of standard gallic acid (calibration range 0-200 µg / ml). The quantitative results obtained are in milligrams equivalent of gallic acid per gram of dry matter (mg GAE / g DM).

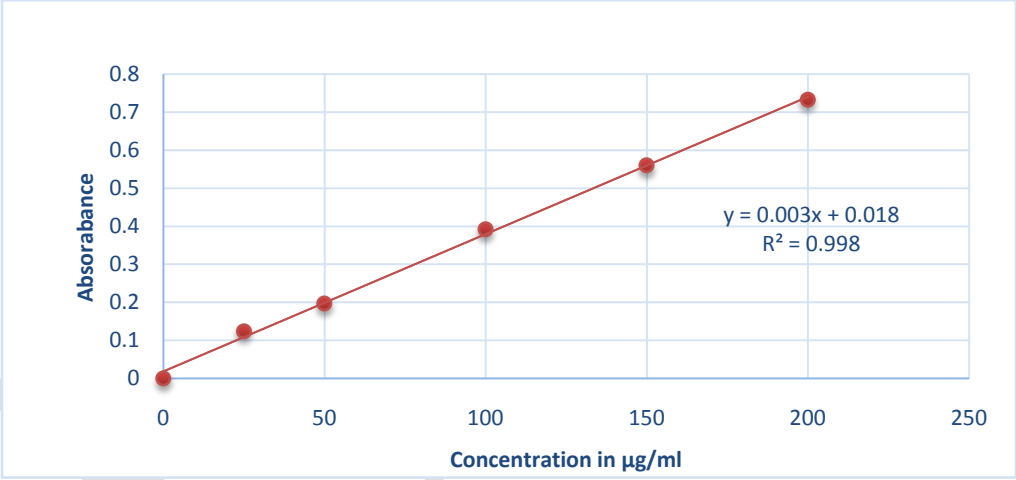

Fig 1: gallic acid Calibration curve

Determination of flavonoid contents

The estimation of the flavonoid content contained in the extracts is determined by colorimetry according to the method of Samatha et al. [26] which relies on the ability of the flavonoids to form a complex with AlCl3 aluminum trichloride. To 0.5 ml of each diluted extract was added 0.15 ml of a 15% sodium nitrite (NaNO 2) solution, allowed to stand for 6 minutes at room temperature, and then 0.15 ml of Solution of 10% aluminum trichloride (AlCl3), after 6 min incubation at room temperature, 2 ml of 4% sodium hydroxide (NaOH) are added and after a last incubation of 15 minutes, the absorbance is measured at 510 nm. The results obtained are expressed in mg quercetin equivalent / g of the dry matter, referring to a calibration curve at different concentrations (0 to 300 µg / ml) of the standard quercetin and using the following equation: A = 0 , 0007 C + 0.0057 with R2 = 0.9953 (Fig 2).

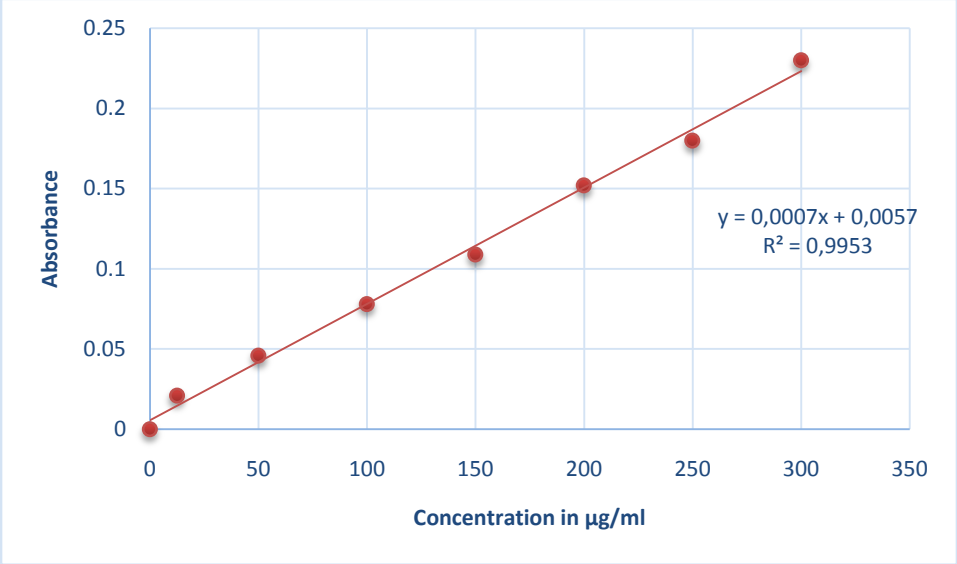

Fig 2: Quercetin Calibration Curve

Determination of condensed tannins

The condensed tannins were determined by oxidation with ferrous sulfate (Fe SO 4, 7H 2 O) in solution in butanol-HCl, as described by Scalbert et al. [27]. 0.5 ml of each extract is mixed with 5 ml of a ferrous sulfate solution (7.7 mg of Fe SO 4, 7H 2 O in 50 ml of HCl / BuOH (2/3)). The tubes were placed in a water bath at 95 ° C. for 15 minutes in the absence of light. The absorbance was read at 530 nm and the results are expressed in milligrams equivalent of cyanidine per gram of dry matter. The content is calculated according to the following formula:

$$\text{mg E Cya/g DM} = \frac{A\ x\ V\ x\ D\ x\ M\ x\ V2}{l\ x\ \varepsilon\ x\ \text{v x m}}$$

With: A is the absorbance of the sample at 530 nm; V is the volume of the total reaction (ml); D is the dilution factor; M is the molar mass of cyanidine (g mol-1); V2: volume of the aqueous extract; L is the path length (cm-1); Ɛ is the molar extinction coefficient (34,700 L mol-1cm-1); V: is 0.5 ml; And m is the mass of dry matter (g).

RESULTS AND DISCUSSION

Spectral Characterization

The Fourier Transform Infrared spectrum was used to identify the characteristic bands of functional groups of the active components based on their peak ratios in the infrared radiation region (Fig 3 and 4). The results revealed the presence of different groups that may be derived from the phytochemicals that form during normal plant metabolic processes (Table 1 and 2).

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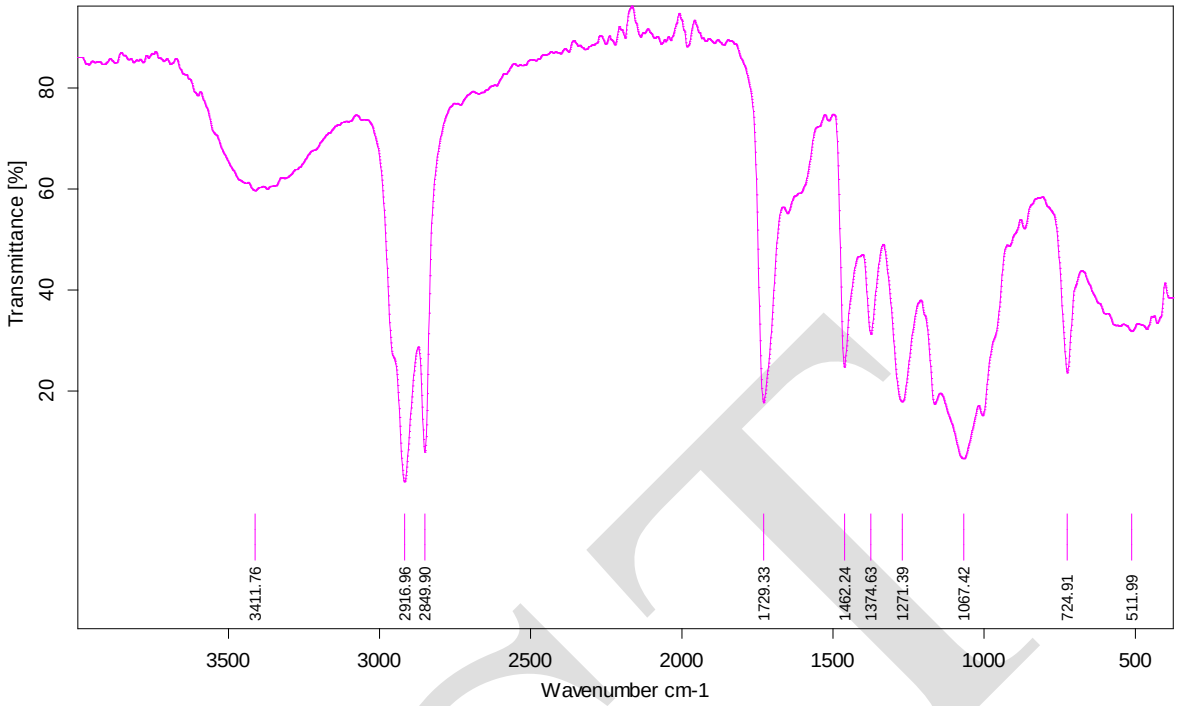


Fig 3: IR SPECTRUM OF DICHLOROMETHANIC EXTRACT

Fig 4: IR SPECTRUM OF METHANOLIC EXTRACT

Table1. Assignments of the IR spectrum groups of the dichloromethanic extract

Bands	Assignments
3411,76 cm^{-1}	Functional groupO-H
2916,96 cm^{-1} et 2849,90 cm^{-1}	Functional group –CH_2 ET –CH_3
1729,33 cm^{-1}	Functional groupC=O
1462,24 cm^{-1}	Functional groupC=C
1374,63 cm^{-1}	Functional groupO-H
1271,39 cm^{-1}	Functional group C=O ET C=C
1067,42 cm^{-1}	Functional group C=O ET C=C

Table 2. Assignments of the IR spectrum groups of the methanolic extract

Bands	Assignment
3448,27 cm^{-1}	Functional groupO-H
2924,33 cm^{-1}	Functional group –CH_2 ET –CH_3
1646,46 cm^{-1}	Functional groupC=O
1372,10 cm^{-1}	Functional groupC-O
1053,32 cm^{-1}	Functional group C=O ET C=C

Mass spectrometry is an analytical technique for detecting and elucidating molecules by measuring their mass. Moreover, it makes it possible to characterize the chemical structure of the molecules by fragmenting them and to carry out quantitative analyzes. The results obtained show that the most abundant compounds for the dichloromethane extract (Fig 5, Table 3) were the saturated and unsaturated fatty acids followed by hydrocarbons and then the tocopherol and finally the squalene for the methanol extract (Fig 6 Table 4), it is especially rich in glycerin followed by saturated and unsaturated fatty acids. These results revealed differences in the profiles between these two extracts and those in the literature that shared only a few substances detected in the essential oils of stems and fruits by GC-MS [22], as well as the volatile Caralluma europaea per head space [23].

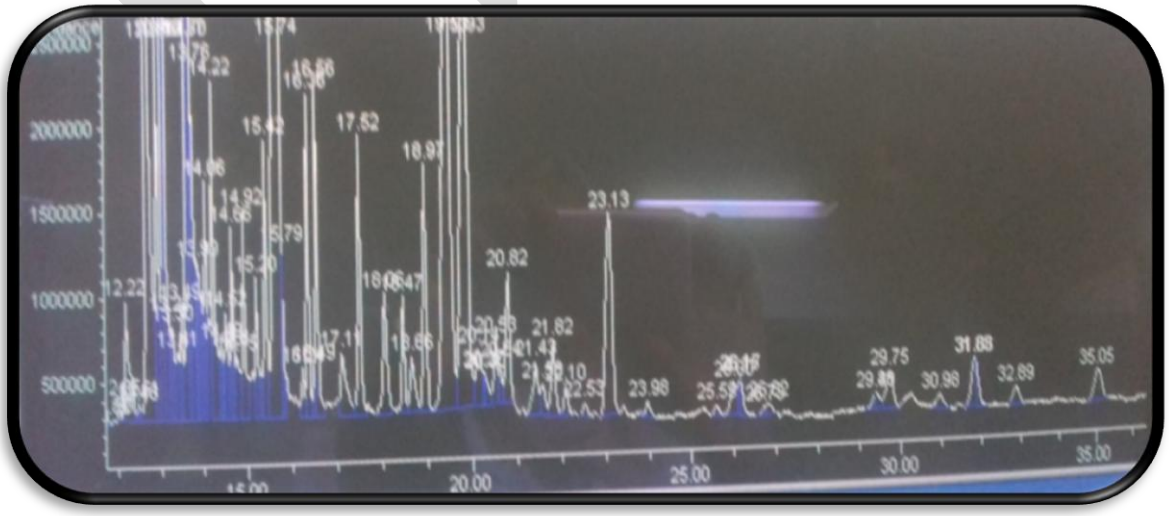


Fig 5: GC-MS Spectrum of dichloromethanic Extract

Table 3. Yield of the main compounds of the dichloromethanic extract

MOLECULES	%
Cis-9-OctadecenoïcAcid (oleicacid $C_{18}H_{34}O_2$) unsaturated acid	3,82%
Hexadecanoic Acid (palmiticacid $C_{16}H_{32}O_2$) saturated acid	3,92%
TetradecanoicAcid (myristicacid $C_{14}H_{28}O_2$) saturated acid	0,17%
9,12-OctadecadienoicAcid (linoleic acid $C_{18}H_{32}O_2$) unsaturated acid	0,09%
Hydrocarbures (hexacosane, heptacosane, docosane, heneicosane et eicosane)	3,43%
Alpha tocopherol vitamine E	0,09%
Squalene (tri terpene)	0,05%

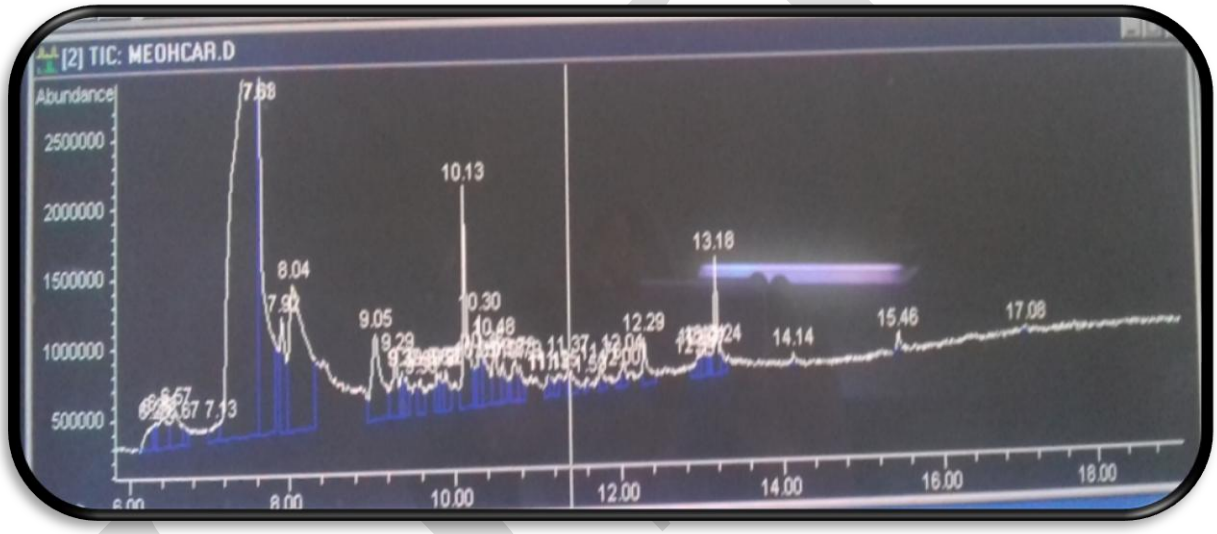


Fig 6: GC-MS Spectrum of methanolic Extract

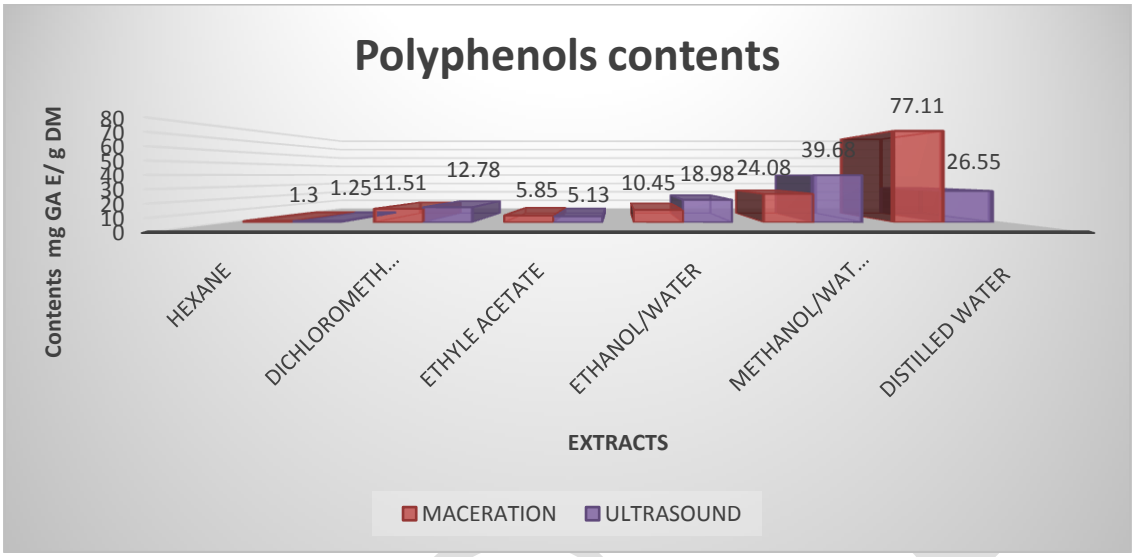
Table 4. Yield of the main compounds of the methanolic extract

MOLECULES	%
Glycerin	2,85%
Hexadecanoicacid (palmiticacid$C_{16}H_{32}O_2$) saturated acid	0,07%
Cis-9-Octadecenoïc acid (oleicacid$C_{18}H_{34}O_2$) unsaturated acid	0,06%

Polyphenols content

The levels of polyphenols in the studied plant obtained by the two methods are shown in graph 1. However, for no polar solvents (hexane, dichloromethane and ethyl acetate) which represent the lowest contents do not reveal a significant difference between two methods, whereas for polar solvents (ethanol, methanol and distilled water) they represent higher grades and a

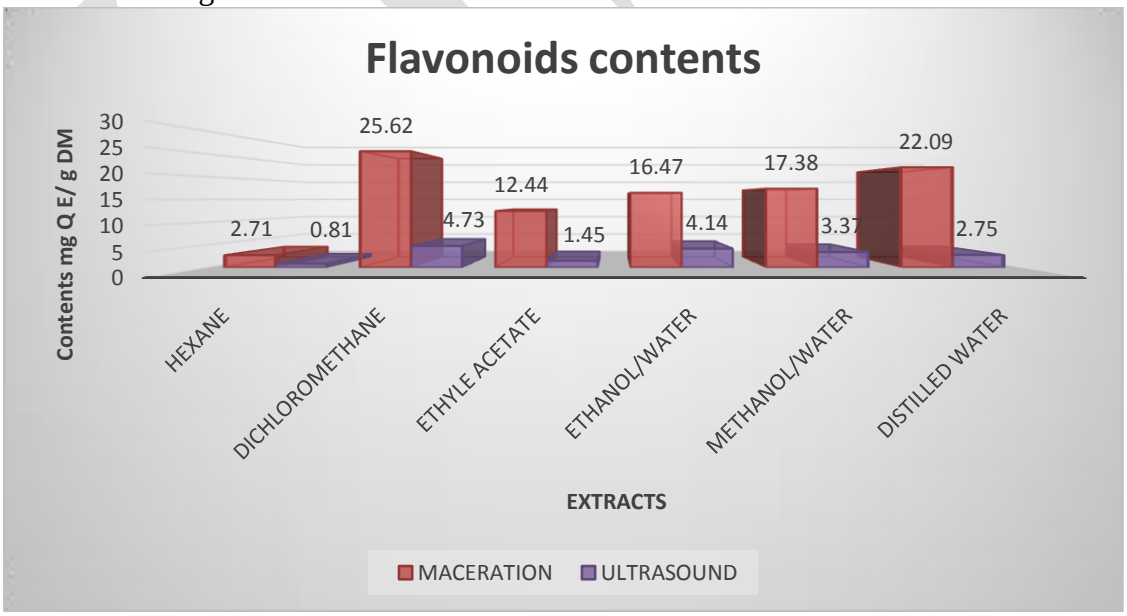
significant difference. Long-lasting maceration appears to be more effective for aqueous extract, unlike ethanolic and methanolic extracts, ultrasound-assisted extraction seems to be more effective.



Graph 1: Polyphenol contents

Flavonoid Content

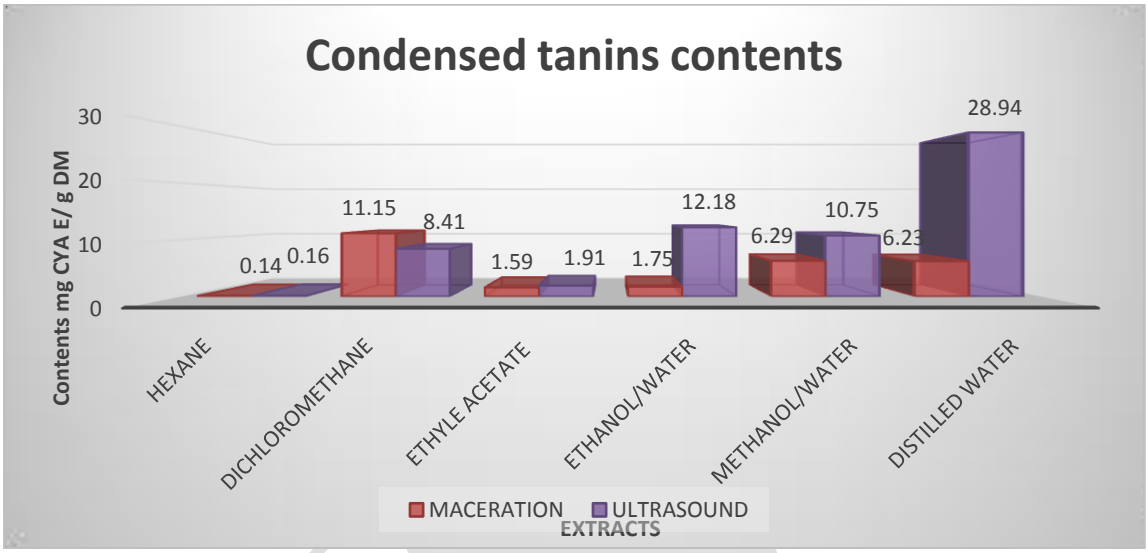
The flavonoid contents obtained by the two methods illustrated in graph 2 reveal a significant difference between the two extraction methods and remain below the levels of the total polyphenols except for the dichloromethane extract obtained by long-term maceration which gives a result (25.62 mg QE / g DM) which can be explained by the fact that the extract may contain compounds having similar chemical structures to pink flavonoids which increases the absorbance of the extract at 510 nm. However, long-term maceration appears to be the best method of extracting flavonoids.



Graph 2: Flavonoid contents

Content of condensed tannins

The interpretation of the results of the condensed tannin content recorded in graph 3 reveals that ultrasonic-assisted extraction is more effective in extracting condensed tannins for all solvents except for the dichloromethane extract, which gives a value Higher for long-term maceration compared to ultrasound assisted extraction. The extraction of condensed tannins generally depends on their chemical nature, the extraction solvent and the operating conditions [28].



Graph 3: condensed tannins contents

CONCLUSION

On the basis of the results obtained, we can conclude that Caralluma europaea is a medicinal species rich in bioactive compounds, which can justify its traditional uses. The contents of these compounds determined by colorimetric method are always very low compared to the yields of the crude extracts; these results indicate that the crude extracts obtained contain compounds other than polyphenols which require further study.

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