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Antioxidant Activity, Anti-Inflammatory and Analgesic Effects of *Caralluma europaea* (Eddaghmouss) in Mice

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Abstract: The aim of the current study is to evaluate the anti-inflammatory, antioxidant and analgesic properties of ethanolic (100 and 200 mg/kg, p.o.) and ethyl acetate extracts (100 and 200 mg/kg, p.o.) of *Caralluma europaea*. Formalin-induced paw licking test, Acetic Acid induced Writhing Test and Hot-PlateTest were used to assess the analgesic activity. Xylene-induced ear edema test was used to evaluate anti-inflammatory activity of those extracts. In this work, the High-Performance Liquid Chromatography technique (HPLC), allowed us to identify and quantify the main phenolic compounds present in ethanolic and ethyl acetate extracts. In vitro anti-oxidant propriety was evaluated using two methods, the 2,2-Diphenyl-1Picrylhydrazyl (DPPH) radical scavenging method and reducing power methods. The main phenols identified were Catechin (24%) and quercetin (18%) in ethanolic extract, while in the ethyl acetate extract, they were quercetin (36%), P-coumaric (30%) and 2-hydroxycinnamic (25%). Analysis of our results had shown that *Caralluma europaea* extracts had exhibited a very potent analgesic activity. Percentage of Pain Inhibition (PPI) in the writhing test, 63.60±4.24% for the Ethanolic Extract (EE) (200 mg/kg, p.o.) and 65.39±3.27% for the Ethyl Acetate Extract (EAE). The PPI of early and late phase in the formalin test were respectively, 41% and 73% for EAE (200 mg/kg; p.o), 28% and 75% for EE. In the hotplate test, latency to the thermal stimuli was increased in a dose dependent manner after the administration of EE and EAE. However, the analgesic potential of EAE seems to be higher than EE. Both EE and EAE presented a significant in vitro redox potential and high anti-inflammatory activity. Our results have shown that *Caralluma europaea* is rich in phenolic compounds and possesses an important antinociceptive, anti-inflammatory and anti-oxidant activity.

Keywords: *Caralluma europaea*, HPLC, Anti-Inflammatory, Analgesic, Antioxidant Activity

Introduction

Inflammation and pain are main preservative responses protecting the organisms counter physical, chemical and pathological alterations (Ezeja *et al*., 2011). The inflammatory response is produced by blood flow changes and escape of cells from the blood into the tissues due to the increased blood vessels permeability (Habashy *et al*., 2005).

Control of pain and inflammation is one of the main therapeutic goals (Ezeja *et al*., 2011). Currently, NonSteroidal Anti-Inflammatory Drugs (NSAIDs) and steroidal drugs are the principal medicament used for the treatment of inflammation and pain. However, several

adverse effects such as liver impairment, manic depression, hypertension, muscle cramps and dizziness, decreased bone density, stomach ulcers, irregular menstruation, vision and allergic diseases are observed after long term use of these drugs (Murugesan and Deviponnuswamy, 2014; Brennan, 1984).

Due to the limited application and adverse effects of synthetic drugs, plant and natural product extracts are now being explored for their therapeutic properties.

Phenolic compounds of plant products are known to possess a wide range of physiological effects such as anti-inflammatory, anti-oxidant, anticancer and antibacterial properties which could constitute a source of therapeutics alternative. Therefore, the assessment of biological properties of plant extracts is of great importance for the discovery of novel drug candidates and validation of traditional medicine.

Caralluma europaea (CE) (or *Apteranthes europaea* Guss.) is a herb from *Apocynaceae* family (Meve and Liede, 2004) and widely distributed throughout Spain, Libya, Tunisa, Algeria and Morocco (Meve and Heneidak, 2005). Several medicinal uses including: Antidiabetic, antihyperglycemic, antiparasitic, antitrypanosomally, antiulcer, neuroprotective, antipyretic, anti-inflammatory, antinociceptive, antioxidant, anti-obesogenic and anti-atherosclerosis activities were associated to various member of *Caralluma* genus properties (Zito *et al*., 2010; Wen *et al*., 2016). However, not all species have been tested for their biological activity. A review of literature of this plant proposed that *Caralluma europaea* has several properties such as antibacterial, antidiabetic and antioxidantactivity (Dutt *et al*., 2012). In addition, the anti-inflammatory and anti-nociceptive potential have never been investigated before.

The present investigation explores the anti-nociceptive, antioxidant and anti-inflammatory properties of *Caralluma europaea* plant extracts using in vitro and *in vivo* methods. The possible mechanism involved in the analgesic and anti-inflammatory activities was also investigated.

Material and Methods

Animals

In this study we used adult male Swiss mice (25-30g). Animals were provided from the Faculty of sciences Semlalia, Cadi Ayyad University. they were housed in cages at room temperature of (25±2°C; 12/12 h light/dark cycle). Mice were fed with standard food and water was offered *ad libitum*. All protocols used in this word respected the approved institutional procedures prescribed in the Scientific Procedures on Living Animals ACT 1986 (European Council directive: 86/609 EEC).

Plant Material

Fresh aerial parts pf CE were collected from the region of Beni Mellal, Morocco in April 2018. Voucher samples representing (CAE 023) were identified by Professor A. Ouhammou and were deposited in the Herbarium of the FSSM, Marrakesh, Morocco

Crude Extract Preparation

Extraction of the dried powdered *Caralluma europaea* aerial parts (100 g) was proceeded by using the maceration method with ethanol (2 L) for 24 h, then filtered and re-extracted again by the ethanol for complete extraction. Extracts were then mixed and evaporated to give ethanol extract (9.2 g). The extract was dissolved in distilled water (100 mL) and transferred to the separatory funnel and portioned by hexane an ethyl acetate (Al-Jadidi and Hossain, 2018). The different extracted portions were dried using a rotary evaporator to give ethanolic and ethyl acetate crude extract (6.1 g).

Acute Toxicity

Mice were randomly grouped into control group and four treated groups (0.5, 1, 2.5 and 5 g/kg body weight) for each extract, containing six animals each. All animals were provided with water *ad libitum*. In order to determine animals' death and toxic effects of changes in general behavior (tachycardia, vomiting, motor activity alteration, cramps, grooming, dyspnea, diarrhea and convulsions) (Shah Ayub *et al*., 1997; Bürger *et al*., 2005).

Assessment of Total Phenolic Compounds, Tannins and Flavonoids Content in Caralluma europaea Extracts

The FolinCiocalteau method was used in order to quantify the total phenolic compound content (Singleton *et al*., 1999). Briefly 20 µL of extract was mixed with 100 µL of Folin-Ciocalteau reagent and 1.16 ml of distilled water, then 300 µL of sodium carbonate solution was added, then the mixture was incubated for 30 min at 40°C. Measurement of mixture absorbance was made at 760 nm. Results were expressed as gallic acid equivalents per Gram of Extract (GAE/g DM). The aluminum trichloride method was used to estimate total flavonoids content. Mixture was prepared using 0.5 mL of extract added to 0.5 mL of 2% $AlCl_3$ ethanol solution, then incubated at room temperature for 1 h before measurement of absorbance at 420 nm. Results were expressed as catechin equivalents per gram of extract (CAT/g DM). In order to quantify Tannins, 100 µL of extract was mixed with 1 mL of (Vanillin-MeOH) 4% and 0.5 mL of HCl. The absorbance was measured at 550 nm after 15 min of incubation at room temperature

(Xu and Chang, 2007). Results were expressed as catechin equivalents per gram of extract (CAT/g DM).

HPLC analysis

High-performance liquid chromatography 5KNEUER) equipped with a (K-1001) pump was used to the identification and quantification of phenolic compounds. 10 mL of Each Extract (EE and EAE) was injected over a C18 (Eurospher II 100-5), column temperature was maintained at 25°C. The column was composed of acidified water (A) and acetonitrile (B). The flow rate was 1 ml/min and the total running time was 60 min. Phenolic compounds identification was done by comparing their retention times with those of standards (Quercetin, Sinapicacid, Narginine, Caffeic acid, Ferulic acid, Rutin ...).

Antioxidant Activity

DPPH Free Radical Scavenging Activity

In order to assess the free radical-scavenging activity of *Caralluma europaea* extracts we used the stable free radical 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), as described by Burits and Bucar (2000). Briefly, 50 µL of different extract concentration were mixed with 2 mL of 60 mM methanol solution of DPPH. Then absorbance of samples was measured at 517 nm after 20 min of incubation in the dark at room temperature. Blank solution was prepared using the same amount of methanol and DPPH. Quercetin and Butylated Hydroxytoluene (BHT) were used as positive control. Calculation of the inhibition of the DPPH (%) was done using the following equation (Badakhshan *et al*., 2012):

$$I\% = \left[A_{blank} - A_{sample} \right) / A_{blank} \left] * 100$$

where, A_{blank} is the absorbance of the control and A_{sample} is the absorbance of the test compound. The sample concentration providing 50% inhibition (IC50) was calculated from the graph by plotting inhibition percentages against sample concentrations.

Reducing Power Assay

As described by Oyaizu (1986), we assessed the reducing power of the EE and EAE. 1 mL of various sample concentrations was added to the phosphate buffer (2.5 mL, 200 mM, Ph 6.6) and potassium ferricyanide (2.5 mL, 1%). The resulted solution was incubated for 20 min at 50°C, after incubation period 2.5 mL of 10% Trichloroacetic Acid (TCA) was mixed with the solution and then centrifuged at 3000 rpm for 10 min. 2.5 mL of the upper layer solution was added to 2.5 ml of distilled water and 0.5 mL of 0.1% ferric chloride ($FeCl_3$). Measurement of coloration formed by the reduction of $Fe3^+$ at 700 nm was used to determine the sample concentration providing 0.5 of absorbance (IC50). BHT, ascorbic acid and quercetin were used as positive controls.

Assessment of Pharmacological Activities

Antinociceptive Tests

Writhing Test

Writhing test is used to evaluate mice response to the chemical nociceptive stimulus (Farouk *et al*., 2008). This test aims to study the peripheral nociceptive sensitivity in mice.

Treatment is carried out 30 minutes before the injection of acetic acid at a dose of 10 ml/kg of the mice weight. Following the intraperitoneal administration of acetic acid (0.6% at 0.1 ml/10g weight) (Farouk *et al*., 2008), animals were placed on plexiglass to observe the number of contortions made by the mice during a period of 30 min.

The Percentage of inhibition (IP) of the nociceptive response is calculated. It is defined as follows:

$$I = \left[\left(NC - NT \right) / NC \right] \times 100$$

Where:
NC: Number of contortions in the control group.
NT: Number of contortions of a tested group.

Formalin Test

In the present study we used the method previously described by De Miranda *et al*. (2001). Briefly, 20 µL of 2% formalin was Subcutaneously injected into the right posterior paw of mice. Each mouse was allowed to adapt the testing box for 5 min prior the formalin injection. nociceptive behavior was quantified as the total time spent in licking and/or biting the injected paw. The quantification of nociceptive behavior was made during two phases (Hunskaar and Hole, 1987) the early nociceptive response normally peaked 5 min after formalin injection and 15-30 min after formalin injection peaked the late nociceptive response representing the tonic and inflammatory pain responses, respectively. 30 min prior formalin injection, mice were pretreated orally with two doses of EE and EAE (100 mg/kg and 200 mg/kg).

Hot Plate Test

This test consists of placing each mouse into a glass cylinder (20 cm of diameter) on a heated surface (55±0.4°C). Treated mice received two doses orally of EE and EAE (100 mg/kg and 20 mg/kg). Negative control group received water orally at 0.1 ml/g. Positive control group had received Morphine intraperitoneally at 10 mg/kg. The latency time taken by the mouse to lick

one of its paws or to jump is noted and considered as the time of the reaction. The duration of this test should not be longer than 20 sec to avoid causing damage to the cutaneous tissue of the mice' paws (Farouk *et al*., 2008).

Anti-Inflammatory Activity

Xylene Induced Mouse Ear Edema

According to Shang *et al*. (2011) Acute inflammation was induced by topical application of 30µl of xylene on the anterior a posterior surfaces of the right ear. Six groups of adult Swiss albino mice were randomly assigned as follow: Control group (Saline solution 0.9%), treated groups with EE and EAE (100 and 200 mg/kg, orally) and a group of diclofenac (10 mg/kg i.p.) were administered 45 min prior to the xylene application. Mice of each group were euthanized 30 min after xylene application and two ear punches (6 mm diameter) were taken from each animal and weighted. Edema was estimated by comparing the weight of the right ear punch with the left ear indicated.

Histological Examination

For histological assessment, six samples of the inflamed ears from the control and treated groups were taken and fixed in formaldehyde (10%). The fixed ear tissues were embedded in paraffin and cut into 3-4 µm sections. The slices were mounted on the glass slides, stained with Hematoxylin and Eosin (HE) for pathological studies as descripted by Kiernan (1999).

Statistical Analysis

Results were shown as means ± S.E.M and the comparisons between the experimental groups were made using one Way ANOVA. The values were considered statistically significant when the P-value was less than 0.05, statistical analyses were done using Sigma Plot 12.5 for Windows.

Results

Acute Toxicity

In this study the evaluation of *in vivo* test on mice for the acute toxicity of the two extract of *C. europaea* showed that the (LD50) is greater than 5 g/kg, in fact no mortality or signs of toxicity and no change body weight wasn't observed during the 48 h following single dose administration (0.5, 1, 2.5 and 5 g/kg body weight) of each of the two *Caralluma europaea* extracts.

Determination of Total Phenolic Compounds, Tannins and Flavonoids

Phytochemical analysis Table 1 showed an important amount of total phenolics, flavonoids and condensed tannins in both extracts studied.

HPLC Analysis

The results of the HPLC technique allowed us to identify and quantify main phenolic compounds HPLC compounds of *Caralluma europaea* ethanolic (EE) and ethyl acetate (EAE). According to the HPLC chromatogram (Fig. 1), the ethyl acetate extract contained rutin, ferulic acid, caffeic acid, narginine, 2-hydroxycinnamic, sinapic acid, P-coumaric acid and quercetin. The principal phenolic components of the ethyl acetate extract are respectively: Quercetin (36 mg EGA/100g DM), P-coumaric (30 mg EGA/100g DM) and 2-hydroxycinnamic (25 mg EGA/100 g DM).

While the *Caralluma europaea*ethanolic extract comprises: Ascorbic acid, catechic acid, vanillic acid, ferulic acid, caffeic acid, narginine, quinin, vanillin, 2-hydroxycinnamic, sinapicacid, catechin, P-coumaric and quercetin (Fig. 2). Catechin was the major phenolic compound identified with (24 mg EGA/100g DM), followed by quercetin (18 mg EGA/100g DM)). The concentrations of the main phenols identified in both extracts are presented in Table 2 and 3.

Table 1: Total phenolics, flavonoids and tannins content of ethanol extract and ethyl acetate extract of the *Caralluma europaea* (Mean ± SEM).

	Ethyl acetate extract	Ethanol extract
Total phenolic contents (mg GAE/g DW)	14.84±0.58	20.93±0.30
Flavonoid contents (mg/g DW)	13.93±0.30	10.19±0.63
Tannin contents (mg CE/g DW)	2.47±0.01	2.28±0.03

Table 2: The phenols amounts found in the ethyl acetate extract ranged from 1 to 37 mg EGA/100 g DM

	Phenolic compounds	Concentrations (mg EGA/100 g DM)
1	Rutin	12,970
2	Furrulic acid	13,908
3	Caffeic acid	15,544
4	Narginine	12,926
5	2-hydroxycinnamic	25,174
6	Sinapic acid	18,296
7	P-Coumaric	30,671
8	Quercitin	36,186

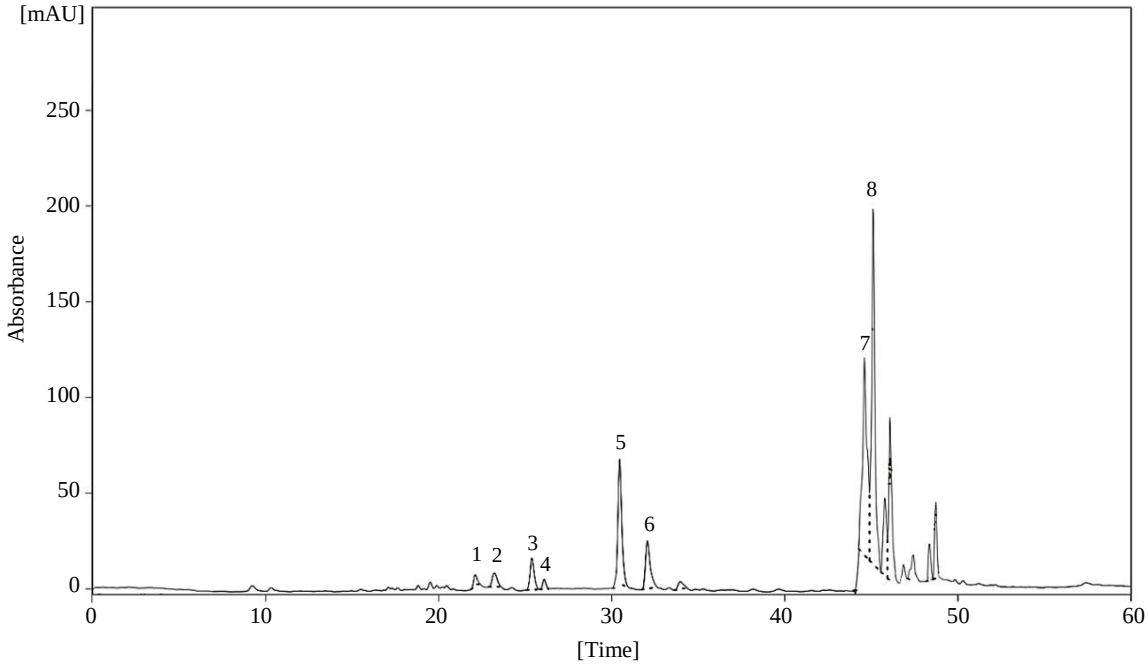


Fig. 1: HPLC chromatogram registered at 280 nm for the major phenolic compounds detected in the ethyl acetate extract of *C. europaea*

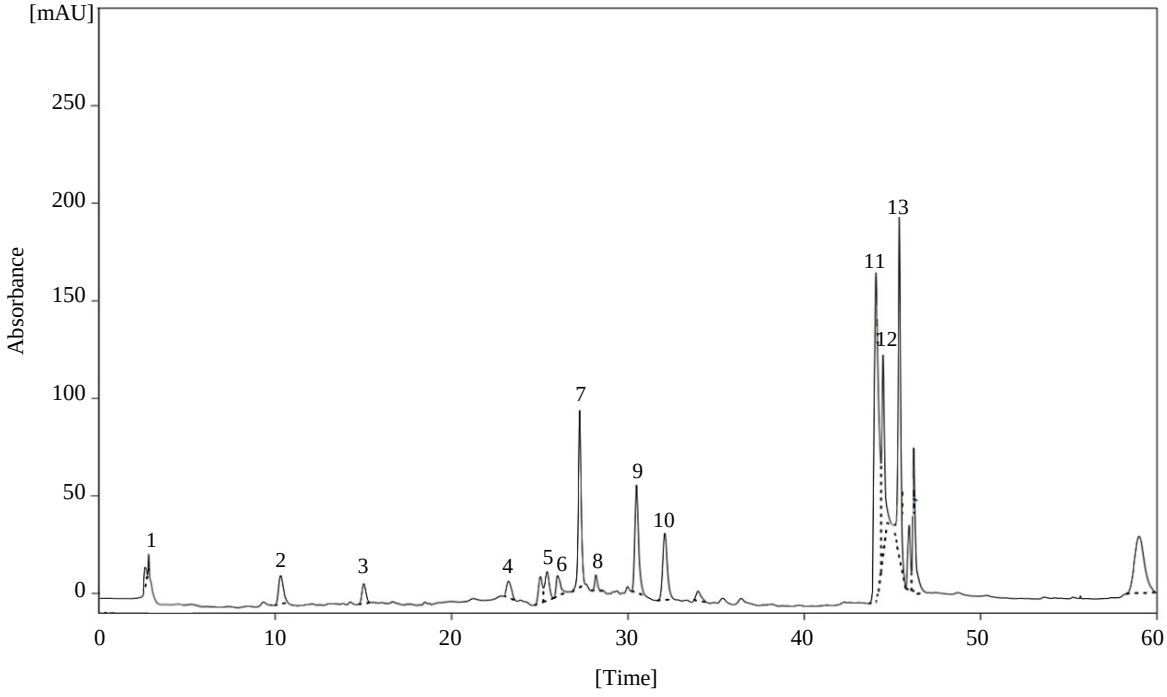


Fig. 2: HPLC chromatogram registered at 280 nm for the major phenolic compounds detected in the ethanolic extract of *C. europaea*

Antioxidant Activity

Caralluma europaea EE and EAE antioxidant activity was evaluated in vitro using two complementary assays: DPPH and reducing power assay.

The concentration that resulted in 50% inhibition (IC50) are shown in Table 4. The antioxidant activities were compared with that of quercetin,

Ascorbic acid and Butylated hydroxytoluene (BHT). Lower IC50 values indicate stronger antioxidant activity.

Table 3: The phenols amounts found in the ethanolic extract ranged from 1 to 25 mg EGA/100 g DM.

	Phenolic compounds	Concentrations (mg EGA/100 g DM)
1	Ascorbicacid	12,488
2	Catechicacid	13,109
3	Vanilicacid	12,657
4	Furrulicacid	12,698
5	Caffeicacid	13,053
6	Narginine	12,743
7	Quinin	15,993
8	Vanilin	12,387
9	2-hydroxycinnamic	15,305
10	Sinapicacid	14,489
11	Catechin	24,797
12	P-Coumaric	16,477
13	Quercitin	18,719

Table 4: IC 50 (µg/ml) values of *Caralluma europaea* ethanolic and ethyl acetate extracts compared to synthetic antioxidants (Ascorbic acid, quercetin and BHT).

	DPPH	Reducing power
BHT	2.39±0.015	2.31±0.016
Quercetin	1.91±0.023	2.66±0.054
Ascorbic acid	1.81±0.015	4.05±0.001
Ethanolic extract	34.60±0.300	79.15±2.110
Ethyl acetate extract	20.60±0.120	54.95±1.040

Table 5: Effect of *Caralluma europaea* ethanolic (EE) and Ethyl Acetate (EAE) extracts on the acetic acid-induced writhing behavior in mice. Ethanolic and ethyl acetate extracts (D1 = 100 mg/kg, D2 = 200 mg/Kg, p.o.) and acetylsalicylic acid (ASA = 200 mg/kg, i.p.) were administered. Results are presented as mean ± SEM ***p<0.001 Vs. Control

Groups	Number of writhes	Percentage of writhes inhibition (%)
Control	145.0±17.78	0±0
ASA (200 mg/kg)	48.2±6.46 ***	66.86±2.84
EE (100 mg/kg)	53.0±2.58 ***	62.04±4.60
EE (200 mg/kg)	51.0±1.76 ***	63.00±4.24
EAE (100 mg/kg)	59.2±2.87 ***	57.05±4.92
EAE (200 mg/kg)	48.6±3.14 ***	65.00±3.27

Both *Caralluma europaea* extracts exhibited significant antioxidant activity, the lowest IC50 was obtained with DPPH for the EAE (IC50 = 20.60±0.12 µg/mL). This activity was less potent than those of reference antioxidants Butylated Hydroxytoluene (BHT), ascorbic acid and quercetin (IC50 values from 1.81±0.015 µg/ml to 4.05±0.001 µg/ml).

Pharmacological Tests

Writhing Test

The result in Our results in Table 5 indicated that the ethanolic and ethyl acetate extract (100 and 200 mg/kg. p.o.) significantly decreased the number of writhes induced by acetic acid ($p < 0.001$). Percentage of writhes inhibition was 63% for ethanolic extract (200 mg/kg, p.o.) and 65% for ethyl acetate extract (200 mg/kg, p.o.) compared with control 0%. ASA caused remarkable pain inhibition (66.8%).

Formalin Test

The intraplantar injection of formalin at 2% generated a reaction typical of two-phase liking. Pre-treatment with EE and EAE (100 and 200 mg/kg, p.o.) significantly reduced both the early and late phases of formalin nociceptive response in a dose dependent manner compared to the control ($p<0.01$; $p<0.001$). Pretreatment with naloxone failed to reverse the analgesic activity of both extracts in this test (Table 6).

Hotplate Test

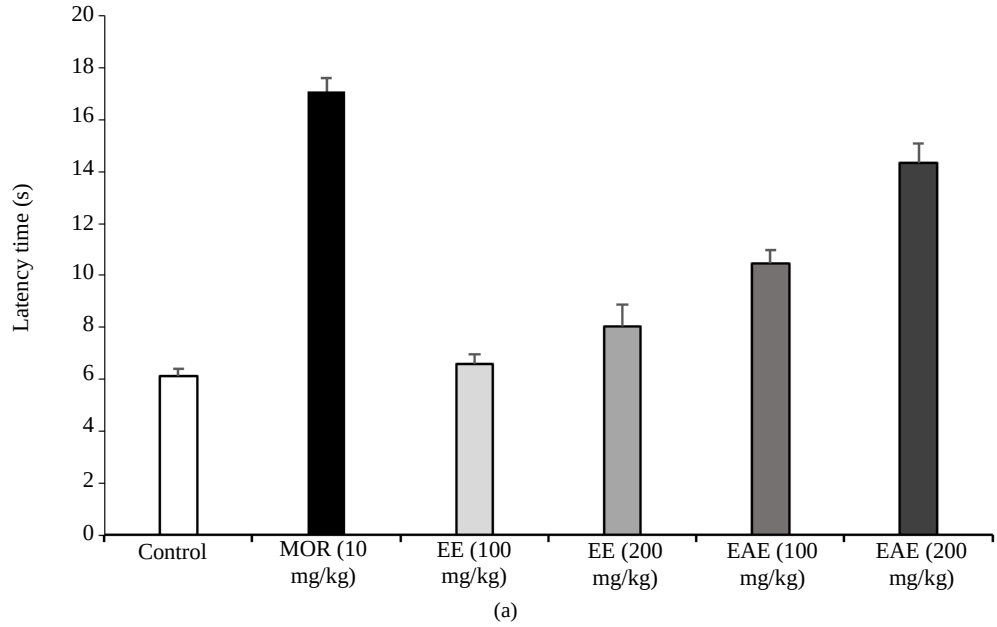
Results of hotplate test showed a highly significant difference between control and EAE ($p < 0.001$). The EE exhibited no effect on the time latency in the hot plate. Compared to the control, EAE have increased latency time, most notably when higher doses were used ($p<0.001$). However, EAE in both doses exhibited a lower analgesic effect compared to morphine (Fig. 3a). The use of naloxone significantly reduced the analgesic effect of morphine and the analgesic effect of EAE was not altered by the use of naloxone (Fig. 3b).

Anti-Inflammatory Activity

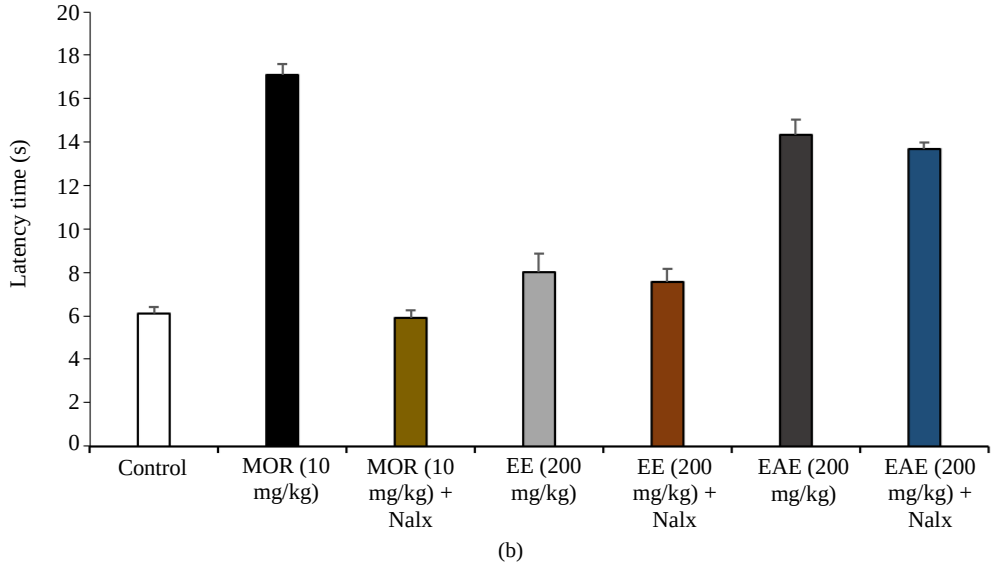
Figure 4 shows the effect of *Caralluma europaea* extracts on the xylene-induced mice ear edema test. After

xylene induction, a significantly increased formation of edema was observed in control mice. The anti-inflammatory activity data indicated that all test concentrations (EE and EAE (100 and 200 mg/kg)) significantly reduced the xylene-induced edema in comparison to control ($p<0.001$), in a dose-dependent manner. However, reduction of the edema by EAE and EE at both doses was less than the standard used (Diclofenac).

As illustrated in Figure 5, histological assessment of the ear tissues showed that the application of xylene induced a marked increase in epidermis thickness, edema and infiltration of Polymorphonuclear (PMN) leukocytes. Oral administration of *Caralluma europaea* extracts (100 and 200 mg/kg) and Diclofenac (10 mg/kg) greatly reduced the indicated changes.



(a)



(b)

Fig. 3: Effect of *Caralluma europaea* ethanolic (EE) and ethyl acetate (EAE) extracts on the nociceptive response in the hot plate test. *Caralluma europaea* extracts (D1 = 100 mg/kg, D2 = 200 mg/Kg, p.o.) were administered 30 min before testing (a). Results of the hot plate test, in absence and presence of naloxone (Nalx) (1 m/kg, s.c.), EE and EAE extracts (200 mg/kg, p.o.) were administered 30 min before testing (b). Results are presented as mean ± SEM ***$p< 0.001$ Vs. Control

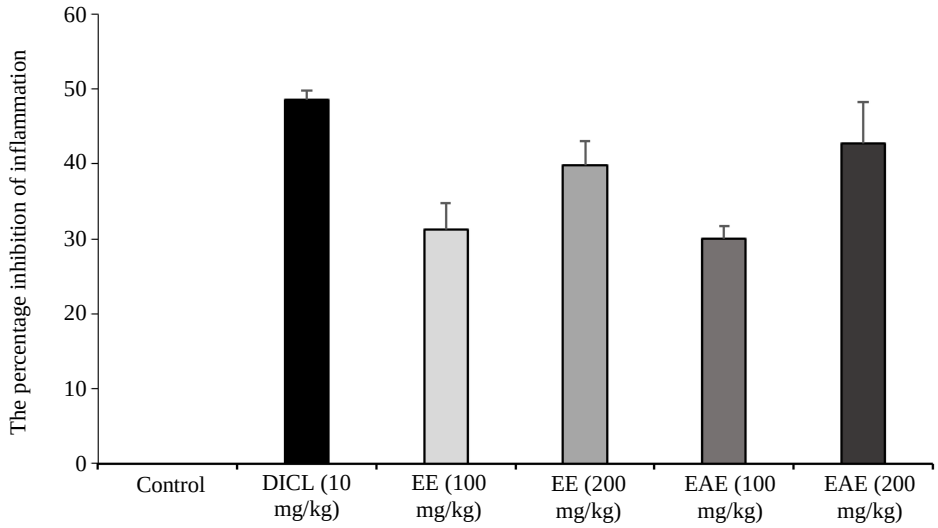


Fig. 4: Effect of ethanolic (EE) and Ethyl Acetate (EAE) extracts on inflammation response in Xylene test. Diclofenac (DICL = 10 mg/kg i.p), ethanolic and ethyl acetate extracts (D1 = 100 mg/kg, D2= 200 mg/Kg, p.o.) were administered 45 min before Xylene application. Results are presented as mean ± SEM

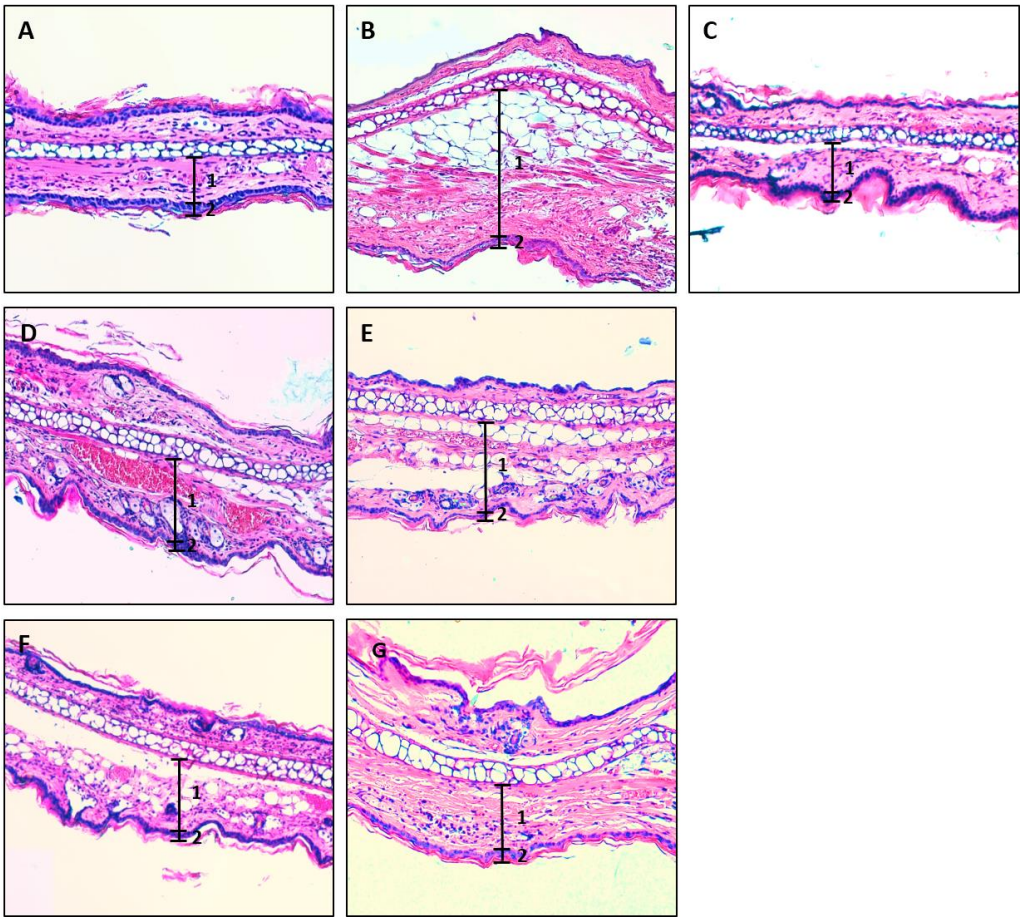


Fig. 5: Histopathological sections showing ear swelling induced by xylene treatment. (A) Normal ear; (B) xylene-induced model control group; (C) treatment with Diclofenac (10 mg/kg); (D) treatment with Ethanolic Extract (100 mg/kg); (E) treatment with Ethyl Acetate Extract (100 mg/kg); (F) treatment with Ethanolic Extract (200 mg/kg); and (G) treatment with Ethyl Acetate Extract (200 mg/kg). 1: Dermal thickness; 2: Epidermis thickness. (HE ×100)

Table 6: The antinociceptive effect of *Caralluma europaea* extracts (EE and EAE), Morphine (MOR), Acetylsalicylic Acid (ASA) and reversal effect of naloxone (Nalx) on formalin-induced pain in mice. Values are represented as the mean ± SEM. Differences between groups were statistically analyzed by ANOVA followed by Tukey's multiple range test. **p<0.01; ***p<0.001 Vs. Control

	Total time spent in licking (s)	
Group	1st phase	2nd phase
Control	122.47±8.84	179.51±5.15
EE (100 mg/kg)	90.54±7.93**	65.77±0,06***
EE (200 mg/kg)	86.83±4.37**	42.16±6.70***
EE (200 mg/kg) + Nalx	87.23±9.50**	44.81±8.87***
EAE (100 mg/kg)	93.34±4.33**	51.974±0.13***
EAE (200mg/kg)	72.26±5.34***	47.33±0.52***
EAE (200mg/kg) +Nalx	73.81±3.43***	57.53±0.53***
MOR (10 mg/kg)	49.91±3.47***	30.01±3.21***
MOR (10mg/kg) +Nalx	134.01±8.40	195.18±10.42
ASA (200mg/kg)	113.83±4.90	39.09±1.61***

Discussion

Our study aims to assess the pharmacological proprieties of *Caralluma europaea*, which are currently used in Moroccan traditional medicine to treat cyst and goiter (Bellakhdar, 1997). Other studies have reported the biological activity of extracts obtained from many species of the genus *Caralluma* such as antihyperglycemic, hepatoprotective, anti-inflammatory and antinociceptive proprieties (Chinenye *et al*., 2013; Shanmugam *et al*., 2013; Abdel-Sattar *et al*., 2013). In our study, to evaluate the analgesic and anti-inflammatory potency of *Caralluma europaea*'s ethanolic and Ethyl Acetate (EE and EAE) extracts. Four experiences were conducted.

Indeed, the tests used for analgesic study are: First, the writhing test was conducted to examine the peripheral analgesic effect. Second, the hot plate test to assess the involvement of central mechanisms, which was known to activate supraspinal and nociceptive spinal pathways (Paulino *et al*., 2003, Arslan and Bektas, 2010). Third, the formalin test was used to explore peripheral and central mechanisms (Tjølsen *et al*., 1992).

In addition, to differentiate between central and peripheral antinociceptive effect of EE and EAE, naloxone was administered with both extracts. Naloxone is an antagonist of the opioid receptors that blocks the central antinociceptive effect of analgesics/opioids.

Usually the acetic acid-induced writhing test is mainly used to examine the antinociceptive activity of biological components (Khan *et al*., 2010; Ibrar *et al*., 2012). The release of several endogenous noxious mediators such as serotonin, histamine, bradykinin and substance P is produced by acetic acid. The contraction of the abdominal muscles is associated with the lengthening of the body and the extension of the forelegs that characterize the pain caused by acetic acid (Ibrar *et al*., 2012). Local peritoneal receptors as well as prostaglandin pathways are assumed to be involved for abdominal contraction (Al-Harrasi *et al*., 2014). In this study, EE and EAE extracts entrained a significant dose-dependent analgesic effect in writhing test. This effect was especially high when EAE administrated at 200 mg/kg dose. The antinociceptive activity observed after oral administration of *Caralluma europaea* EE and EAE can occur because of blocking of prostaglandin pathways or inhibition of endogenous mediators.

The hot-plate test is a pain test in which opioid or central analgesics apply their analgesic effects via supraspinal and spinal receptor (Abbott and Melzack, 1982). Results of this test show that, morphine group present a significant antinociceptive effect. Oral administration of EAE extracts also a strong analgesic effect in this model. However, the use of naloxone has completely reversed the antinociceptive effect of morphine, while antinociceptive action of EAE was not reversed by the opioid antagonist, naloxone. So, the mechanism (s) underlying the antinociceptive action of the extract in both hot-plate and formalin tests appear to be unrelated to the opioid system.

In order to confirm the antinociceptive activity of our extracts, the formalin test was selected. Results showed that the time spent in licking the injured paw was significantly reduced by oral administration of EE and EAE in both phases. The EAE was highly efficient at 200 mg/kg dose than the EE. Our results show also that peripheral acting drug, acetylsalicylic acid, reduce nociception only in the late phase by inhibiting the inflammatory process (Hunskaar and Hole, 1987; Rosland *et al*., 1990). These results suggest that EE and EAE have analgesic action at the central and peripheral levels.

The possible antinociceptive activity may be linked to the high polyphenol content of plants, especially flavonoids and tannins (Handa *et al*., 1992; Orhan *et al*., 2007). Whose were noted in our study (Table 1). It is well established that flavonoids have an analgesic activity on the acetic acid-induced writhing test

(Calixto *et al*., 2000; Ahmed *et al*., 2007). The analgesic effect mechanism of the extracts in our study can be related to the process implicated in inhibiting nociceptor sensitization, blocking nociceptors (peripheral and/or central) or regulating sensitized nociceptors (Ferreira *et al*., 1990). Some biochemical studies on the mechanism of action of flavonoids have revealed that these compounds can block a large range of enzymes implicated in the synthesis of prostaglandins. The polyphenols found in this plant, such as quercetin, can be responsible for the analgesic activities observed.

The acute inflammation induced by xylene in the mouse ear has been used in general as a classic method to detect the effects of anti-inflammatory agents (Hossein Zadeh and Younesi, 2002; Kou *et al*., 2005). The study in this paper examined the impacts of *Caralluma europaea* EE and EAE on acute xylene-induced inflammation. After topical application of xylene, significant increases in ear weight were detected due to the acute inflammatory response; these increases in ear weight were used as valuable indicators for anti-inflammatory effects (Al-Majed *et al*., 2003; Ojewole, 2005). In this study, the increase in ear weight was inhibited on a dose-dependent basis by pre-treatment with our extracts and Diclofenac (Non-Steroidal Anti-Inflammatory Drug). Histopathologically, severe vasodilation, inflammatory cell infiltration and edematous skin changes were detected as signs of acute inflammation after application of xylene (Puerta *et al*., 1996; Kou *et al*., 2003; Rotelli *et al*., 2003); as a consequence of these histopathological changes, ear tissue thickness was markedly increased. However, there were dose-dependent decreases in these histopathological changes and ear tissue thickness after pre-treatment with *Caralluma europaea* EE and EAE extracts. This inhibition has been considered as direct evidence that the two extracts used in this study have positive effects on the reduction of the acute inflammatory response, comparable to those of Diclofenac. The known anti-inflammatory action of this NSAID is the inhibition of the cyclooxygenase activity (Miura *et al*., 1993; El-Banhawy *et al*., 1993; Mc Cafferty *et al*., 1995; Waterbury *et al*., 2006). This was confirmed by the observed reductions in weight and skin thickness and by the improvement of several histopathological indicators.

The anti-inflammatory activity of our extracts could be attributed to its phytochemical compounds such as polyphenols. Several plants rich in polyphenols, particularly flavonoids, are believed to have important pharmacological activities, such as antioxidant, anti-inflammatory, muscle relaxant and antinociceptive actions (Meotti *et al*., 2006; Fernandez *et al*., 2009).

Furthermore, in several studies, flavonoids like rutin, quercetin, sinapic acid, p-coumaric acid, 2-hydroxycinnamic and catechin yielded important anti-inflammatory and analgesic activities (Nagasaka *et al*., 2007; Nakanishi *et al*., 2010; Ramesh *et al*., 1998; Bittar *et al*., 2009; Farahpour, 2014). Accordingly, HPLC analysis had shown high amounts of these phenolic components especially in the *Caralluma europaea* EAE extract.

Numerous reports indicate that antioxidants are able to reduce pain and inflammation produced by chemical and thermal excitation (Hacimuftuoglu *et al*., 2006). In normal conditions, there is an equilibrium of the free radical–antioxidant system. However, the impairment in the free radical–antioxidant profile induces cellular and tissue damage known as oxidant damage (Karaca *et al*., 2006). In our study, the protective role of *Caralluma europaea* EAE and EE against oxidative stress was evaluated by using DPPH free radical-scavenging activity and reducing power assay. Our results are in line with the results reported by Ait Dra *et al*. (2018) on the methanolic extract of *Caralluma europaea.*

Reducing power is an antioxidant mechanism of action and can serve as an indicator of the potential antioxidant activity of new drugs (Jayaprakash *et al*., 2001). Many studies have demonstrated that the antioxidant effect is linked to the development of reductions (Yen and Duh, 1994). Thus, in the present study, the antioxidant activity of *Caralluma europaea* extracts can be combined with its reducing power.

According to Rekka and Kourounakis (1991), the scavenging of DPPH radical is related to the lipid peroxidation inhibition. In addition, the work of Kaviarasan *et al*. (2007) showed that the DPPH radical involves a process of transferring hydrogen atoms. in this assay, the antioxidant activity on DPPH radical of EAE and EE extracts can be attributed to a direct role in trapping free radicals by donating hydrogen atom.

This study brought a demonstration of antioxidant, analgesic and anti-inflammatory activities by *Caralluma europaea* ethanolic and ethyl acetic extracts. Although, to date, the precise mechanism underlying the antinociceptive and anti-inflammatory action of *Caralluma europaea* remains unclear, this plant, rich in bioactive polyphenols might be of potential interest in the future development of new clinically relevant drugs for the management of pain and inflammation.

Conclusion

Our study revealed that complementary analgesic and anti-inflammatory activities of the ethanolic and ethyl acetic extracts of *Caralluma europaea*, can be related and mediated by its antioxidant activity. This justifies at least the ethnomedical uses of this plant. Although they lack other studies, such as *in vivo* antioxidant activity and chronic toxicity studies, this plant could have a potential interest in the development of new drugs for pain and inflammation management.

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Authors Contributions

All authors participated in all experiments, coordinated the data-analysis and contributed to the writing of the manuscript.

Conflict of Interest

The authors report no declarations of interest.

Ethics

This article is originally from the authors works. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

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