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ANTISPASMODIC EFFECT OF *CISTUS LADANIFERUS* ESSENTIAL OIL ON ISOLATED RODENT JEJUNUMS

Mohammed Aziz^{1*}, Ahmed Karim¹, Naoual Tab¹, Mohammed Berrabah², Hassane Mekhfi¹ and Ahmed Melhaoui³

^{1*}Laboratory of Physiology and Ethnopharmacology, Sciences Faculty, Mohammed the First University, BP 717, 60000, Oujda, Morocco.

²Laboratory of Mineral Solid and Analytical Chemistry, Sciences Faculty, Mohammed the First University, BP 717, 60000, Oujda, Morocco.

³Laboratory of Organic Chemistry, Macromolecular and Natural Products, Sciences Faculty, Mohammed the First University, BP 717, 60000, Oujda, Morocco.

ABSTRACT

The essential oils were obtained by hydro-distillation from aerial parts, leaves and flowers of *Cistus ladaniferus* (EOCL). They were analyzed for their chemical composition by GC/MS. Twenty six compounds, representing 93.03 % of the oil were identified, with cubenol (25.88%), viridiflorol (13.90%), α -cadinene (13.49%) and Camphene (5.44%) being the major constituents. The EOCL showed concentration-dependent (2-30 ng/ml) and reversible inhibition of spontaneous movements of rabbit jejunum. Submaximal contractions induced by 60 mM KCl or Carbachol were concentration dependently inhibited by EOCL with IC_{50} value of 6.25 ± 0.61 ng/ml and 50.02 ± 0.2 ng/ml respectively. The concentration contraction curves to $CaCl_2$ and Carbachol in rat jejunum were displaced to the right by EOCL in a non-competitive manner, with a depression of the maximum contractile response. The antagonism they exert on CCh, and KCl above mentioned contractions seems to be functional, because it is not specifically directed toward any particular receptor.

KEYWORDS

Cistus ladaniferus, Essential oil, Jejunum, Antispasmodic and Smooth muscle.

Author for Correspondence

Pr. Mohammed AZIZ,
Laboratory of Physiology and Ethnopharmacology,
Sciences Faculty,
Mohammed the First University, BP 717, 60000,
Oujda, Morocco.

Email: azizmo5@yahoo.fr.

INTRODUCTION

Cistus ladaniferus L., known commonly as rock-rose, is a medicinal plant originated from Mediterranean area¹. This strong aromatic shrub is widely used in herbal medicine by local population of the North-east of Morocco as anti-diarrhoeal and antispasmodic. Moroccans call it "Touzal". This plant (aqueous extract) exerts different pharmacological effects such as an antiaggregant², antihypertensive³ and an antioxidant effects^{4,5}.

Essential oil, absolute and resinoid of this plant had been shown to exhibit antifungal and antibacterial effect⁶ and it is used in cosmetic industry. The essential oil extraction and composition are well documented in the literature^{7,8,9}.

In continuation of our previous study on the aqueous extract from *Cistus ladaniferus* L.¹⁰, we aimed to investigate the effects of essential oil from this plant on the isolated smooth muscle of rat and rabbit jejunum to evaluate their popular use as spasmolytic and their possible mechanism of action.

MATERIALS AND METHODS

Plant material

Cistus ladaniferus L. was collected locally during the flowering period (in May) from the north eastern area of Morocco, and botanically identified by Professor B. Haloui at the Department of Biology, Faculty of Science, Mohammed the First University, Oujda, Morocco. A voucher specimen (N^o rab 502/63) had been previously deposited with the Scientific Institute of Rabat.

Extraction of essential oil

Air-dried aerial parts, leaves and flowers (100 g) of *Cistus ladaniferus* were hydro distilled for 3 hours using a Dean stark apparatus to yield essential oil 1.2 %. The extracted oil was stored at +4°C. It was made up as 500 µl/ml stock solution in DMSO (< 1%).

Gas chromatography-Mass Spectrometry (GC-MS)

The essential oils were analysed by gas chromatography/mass spectrometry using the Trace GC Ultra/Polaris Q system. A VB5 column (30m x 0.25mm inner diameter, 0.25 µm film thickness) was used with helium as the carrier gas (1 ml/min). The GC oven temperature was kept at 50 °C for 5 min and programmed to increase to 250 °C at a rate of 4 °C/min, then to be kept constant at 250 °C for 3 min and then to increase to 300 °C at a rate of 25 °C/min. Split flow was used; and the injector temperature was held at 250 °C. MS were taken at 70 eV. Mass range was from m/z 20 to 350, temperature of interface was 300 °C. A library search was carried

out using both the “Wiley GC/MS Library” Nist and PMW (Pfleger Maurer Weber).

Animals

Male Wistar rats (250–300 g) and male New Zealand rabbits (1.5-2 kg) were housed in the Experimental Animal House of the Department of Biology, Oujda sciences faculty, in standard environmental conditions. They were fed with rodent diet and water ad libitum. Animals had free access to water but food was withdrawn 24h prior to experiment. All animals were cared for in compliance with the Guide for the Care and Use of Laboratory Animals, published by the US National Institutes of Health (NIH Publication 85-23, revised 1996; see <http://grants.nih.gov/grants/olaw/olaw.htm>).

Solutions and Drugs

The following solutions were used

- Normal Krebs-Henseleit Bicarbonate (KHB) solution composed of (mM) NaCl, 118; KCl, 4.7; CaCl₂, 2.5; MgSO₄, 1.2; NaHCO₃, 25; KH₂PO₄, 1.2 and glucose 10.
- High K⁺ KHB (75mM) solution composed of (mM), NaCl, 48; KCl, 75; CaCl₂, 2.5; MgSO₄, 1.2; NaHCO₃, 25; KH₂PO₄, 1.2 and glucose 10.
- Calcium-free high K⁺ KHB (75mM) solution composed of (mM), NaCl, 48; KCl, 75; CaCl₂, 0.0; MgSO₄, 1.2; NaHCO₃, 25; KH₂PO₄, 1.2 and glucose 10.
- Calcium-free KHB solution composed of (mM), NaCl, 121.7; KCl, 4.7; CaCl₂, 0.0; MgSO₄, 1.2; NaHCO₃, 25; KH₂PO₄, 1.2 and glucose 10, made up in distilled water, the pH was adjusted to 7.4.

The following drugs were used for the experiments: Carbamylcholine chloride (Carbachol, CCh) and dimethyl sulfoxide (DMSO) were purchased from Prolabo, papaverine hydrochloride from Fluka and verapamil hydrochloride, propranolol hydrochloride, prazosin hydrochloride, and yohimbine hydrochloride from Sigma.

Experimental procedure

The spasmolytic activity of the essential oil was studied using isolated jejunum preparations from rabbits and Wistar rats. The animals were sacrificed by cervical dislocation. Segments of jejunum (2 cm)

were removed and mounted in 10 ml organ baths containing Krebs-Henseleit buffer (KHB). The bath solution was maintained at 37°C, pH 7.4 and gassed continuously with air bubbling. A 60 min equilibration period was allowed during which the physiological solution was changed every 15 min. EOAM was dissolved in DMSO (1%) and added to the organ bath. Each concentration of the essential oil was in contact with the tissue for at least 7 min before its effect was evaluated.

Relaxing activity on isolated rabbit jejunum

On isolated jejunum rats, the activity of *Cistus ladaniferus* essential oil was tested in the absence and presence of antagonist adrenergic receptors yohimbine, prazosin and propranolol at 5×10^{-5} M. these antagonists are added at the same time.

Relaxant effect on K⁺ and Carbachol induced contractions

The jejunum of rats was contracted with K⁺ (60 mM) and Carbachol (10^{-6} M) to a maintained tone, at this point cumulative doses of essential oil were added to the tissue bath. Verapamil and papaverine are used like control respectively.

Inhibition of dose-response curves to Carbachol (Cch) and CaCl₂

After stabilization period of 60 min in KHB, cumulative concentration-response curves were obtained for Cch (10^{-8} - 10^{-6} M) and CaCl₂ (0.1-10 mM), in the absence or presence of the essential oil.

Statistical analysis

The results are expressed as mean \pm S.E.M. The statistical analysis was obtained by the Student's test and P < 0.05 was considered to be significant.

RESULTS AND DISCUSSION

The composition of EOCL was analysed by GC/MS. Twenty six compounds, representing 93.03% of the oil were identified, with cubenol (25.88%), viridiflorol (13.90%), α -cadinene (13.49%) and Camphene (5.44%) being the major constituents (Figure No.1, Table No.1). α pinene (0.86%) is not very abundant in comparison with other compounds of oil obtained from *Cistus ladaniferus* by other authors⁹. Generally constituents vary on season, weather, soil and geography locality. These

constituents vary also by the technique used. EOCL constituents have been isolated by steam distillation^{11,12}, hydrodistillation^{7,13} and also by solvent extraction⁸. All these techniques have advantages and drawbacks⁹.

EOCL caused concentration-dependent (2 ng/ml-30 ng/ml) relaxation of spontaneous contractions of isolated rabbit jejunum preparations with a 100% relaxation at 30 ng/ml. This effect was reversible after 30 min washing. To check whether the oil acts on adrenergic receptors, we added the 3 inhibitors, prazosin, propranolol and yohimbine, at the same time in the organ bath, to block α_1 , α_2 and β adrenergic receptors respectively. The relaxing effect of essential oil of *Cistus ladaniferus* is unlikely to depend on the stimulation of the α_1 , α_2 and β adrenergic receptors because it was not blocked by these inhibitors.

The contraction of the jejunum smooth muscle is dependent upon an increase in the cytoplasmic free Ca⁺⁺, which activates the contractile elements¹⁴. The spasmolytic effect of the medicinal plants is usually mediated through calcium channel blockade¹⁵. To see whether the spasmolytic effect of *Cistus ladaniferus* is also mediated through the same mechanism, the essential oil was tested on high K⁺ (60 mM) induced contractions. K⁺ is used to depolarize the membrane of isolated tissue cells and in turn produced sustained contractions which depend on flux of Ca⁺⁺ into the cell through L-type voltage-operated channels (VOC)¹⁶. A substance which inhibits K⁺ induced contractions is considered as a calcium channel blocker. EOCL in a concentration-dependent manner inhibited the rat jejunum tonic contractions produced by 60 mM K⁺ with IC₅₀ value of 6.25 ± 0.61 ng/ml (Figure No.2). Thus this inhibition may be considered as an outcome of restricted Ca⁺⁺ entry via voltage dependent Ca⁺⁺ Channels. Verapamil, a VOC inhibitor used as positive control, at 10^{-5} M produced a 100% relaxation effect. In short, calcium ions gain access to the cytoplasm through voltage-activated or receptor-operated calcium channels¹⁷. Furthermore, pre-treatment of the tissue with EOCL (50, 70 and 100 ng/ml) shifted the CaCl₂ curves to

the right (Figure No.3). These results confirm the calcium channel blocking activity of the test substances. The same results were obtained by Gilani et al^{15,16}.

EOCL inhibited submaximal rat jejunum contraction induced with CCh, in a concentration-dependent manner (Figure No.4) with IC₅₀ value of 50.02± 0.2 ng/ml. It had also an inhibitory effect on the concentration-response curve produced by CCh by reducing the maximum induced contraction (Figure No.5). Papaverine, used as positive control, at 10⁻⁵ M produced a 100% relaxation effect mainly via the intracellular accumulation of cAMP and/or cGMP, by inhibiting phosphodiesterase, by having effects on Ca⁺⁺ movement and inhibition of mitochondrial respiration^{18,19}. CCh, an analogue of acetylcholine, stimulates membrane bound cholinergic receptors, which in subsequent steps leads to increase in intracellular Ca⁺⁺ ion concentration and contraction of the muscle²⁰. This mechanism tends to suggest that effect of EOCL is mediated through muscarinic receptors like a noncompetitive antagonism attenuating the maximum response^{15,20}. There are

reports about antispasmodic activity of aqueous extract of other cistacea species such as *Cistus incanus*, *Cistus monspeliensis*²² and *Cistus populifolius*²³. In our earlier studies¹⁰ we have observed similar inhibitory effects of aqueous extract of *Cistus ladaniferus* on rat and rabbit intestinal smooth muscle contractions. There are also reports about antispasmodic activity of other plants essential oil^{24,26}. The observed final pharmacological activity of essential oil *Cistus ladaniferus* may be due to an unspecific “reactive compound” and/or to the combined effects of the several chemical constituents of the plant. One or more components of EOCL could act on the plasma membrane, the muscarinic receptors, the VOCs or one step of intracellular pathways that contribute to the contraction of smooth muscle cells. Probably this spasmolytic activity of EOCL may be, due to the presence of Camphor, terpinene²⁴, α and β pinene²⁶, Borneol and azulene which have been reported to be smooth muscle relaxants. However, the presence of other spasmolytic compound(s) cannot be excluded.

Table No.1: Constituents of *Cistus ladaniferus* L. essential oil

S.No	RT ^a	Compound	Area %
1	8.35	α-pinene	0.86
2	8.83	cis-Ocimene	1.54
3	9.38	Camphene	5.44
4	12.14	α-Terpinene	0.45
5	13.82	γ-Terpinene	1.18
6	17.00	Camphor	0.98
7	17.87	Borneol L	3.71
8	18.31	3-Cyclohexen-1-ol	1.79
9	24.39	α-Cubebene	1.32
10	24.96	1,2,4-Metheno-1H-indene	1.49
11	25.70	Germacrene-D	1.28
12	28.32	δ-Cadinene	1.21
13	28.87	Germacrene-D	1.96
14	29.61	α-Guaiene	0.67
15	29.82	α-Cadinene	13.49
16	30.37	α-Calacorene	0.82
17	31.13	Palustrol	0.63
18	31.54	Caryophyllene oxide	3.61
19	31.83	Veridiflorol	13.90
20	32.19	Cubenol	25.88
21	32.85	Isoledene	4.16
22	33.26	α-Eudesmol	2.49
23	34.12	Azulene	1.27
24	40.04	Pentacosane	1.62
25	42.74	1H-Naphtho[2,1-b]pyran	0.44
26	43.31	Kaur-16-ene	0.84

^a RT: Retention time.

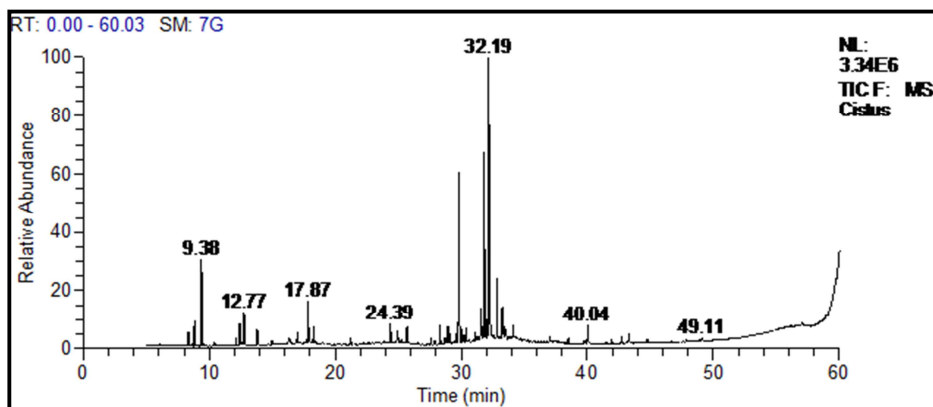


Figure No.1: Typical chromatogram of the essential oil from aerial part of *Cistus ladaniferus L*

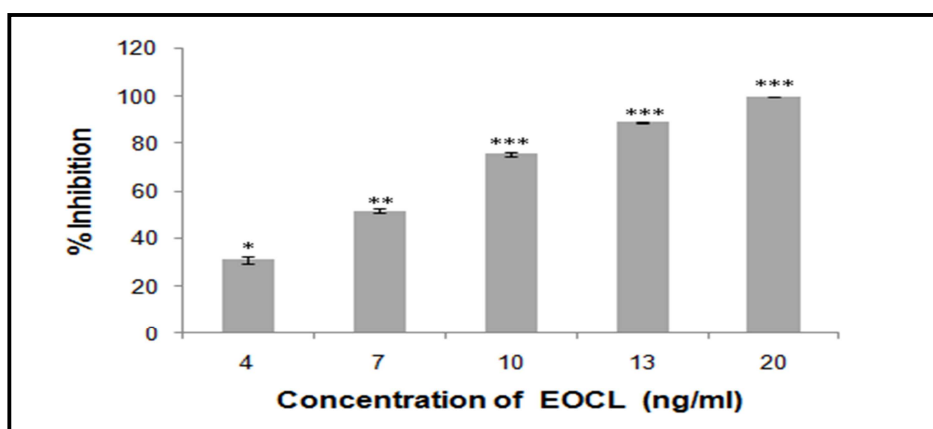


Figure No.2: Effect of essential oil of *Cistus ladaniferus* in tonic contraction induced by high potassium depolarizing solution. (mean \pm S.E.M, n=6). *, $P<0.05$; **, $P<0.01$ and ***, $P<0.001$ were statistically significant difference from control

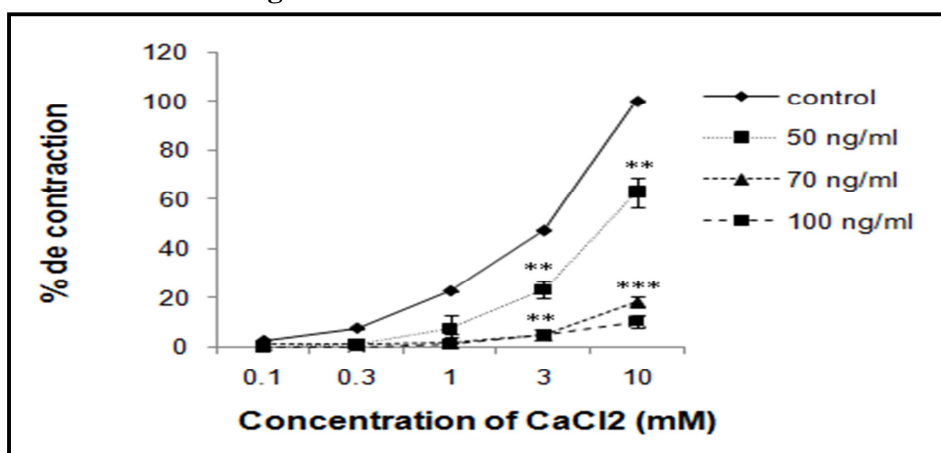


Figure No.3: Comparison of the dose-response curves of CaCl_2 in the absence and presence of different concentration of the essential oil *Cistus ladaniferus* in isolated rat jejunum (mean \pm S.E.M, n=6). *, $P<0.05$; **, $P<0.01$ and ***, $P<0.001$ were statistically significant difference from control

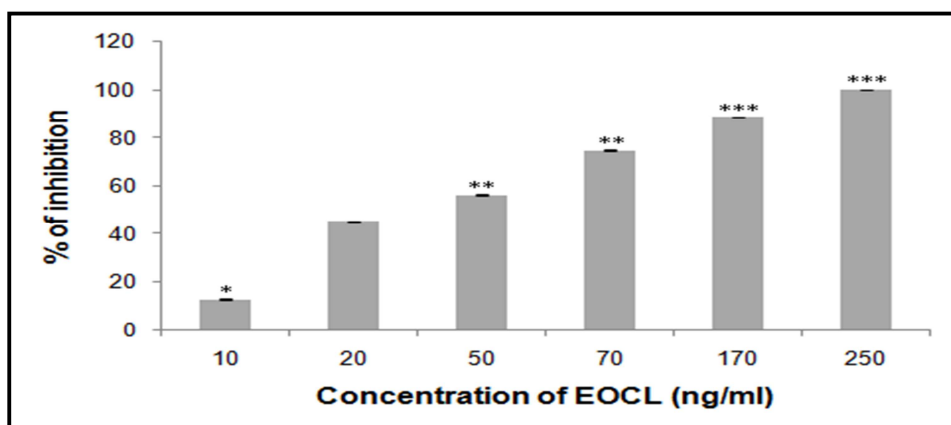


Figure No.4: Effect of essential oil of *Cistus ladaniferus* in tonic contraction induced by Cch (10-6) depolarizing solution (mean \pm S.E.M, n=6). *, $P<0.05$; **, $P<0.01$ and ***, $P<0.001$ were statistically significant difference from control

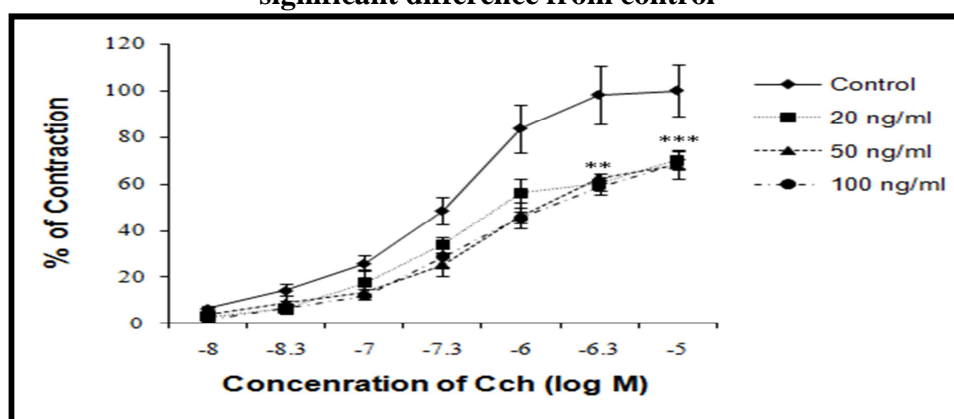


Figure No.5: Comparison of the dose-response curves of Cch in the absence and presence of different concentration of the essential oil *Cistus ladaniferus* in isolated rat jejunum (mean \pm S.E.M, n=6). *, $P<0.05$; **, $P<0.01$ and ***, $P<0.001$ were statistically significant difference from control

CONCLUSION

Cubenol, viridiflorol, α -cadinene and Camphene are identified as major constituents of the essential oil of *Cistus ladaniferus*. Results from testing of antispasmodic activity of this oil justify the use of *Cistus ladaniferus* in folk medicine as a remedy for gastrointestinal disorders. Probably this spasmolytic activity may be, due to the presence of the minor constituents like Camphor, terpinene, α and β pinene), Borneol and azulene which have been reported to be smooth muscle relaxants. However, the presence of other spasmolytic compound(s) cannot be excluded. Like this oil had antioxidant, antifungal and antibacterial activities, it represents a great potential in anti-cancer treatments and certainly deserve further study.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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