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Full Length Article

Anti-hemolytic and Anti-cytotoxic Effect of Two *Artemisia* Species (*A. campestris* and *A. herba-alba*) Essential Oil against Snake Venom

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Abstract

In this study, we verify the possible activity of *Artemisia herba-alba* and *A. campestris* (Asteraceae) essential oil against the snake venoms. The leaves of *A. campestris* has been used in the folk medicine against snake moisture. The aerial part extracts of both plants have been tested for their action against snake venom. Interaction of *A. campestris* (E1) and *A. herba alba* (E2) essential oils with proteins extracted from snake venom has been tested. Lethality in mice, inflammation and cytotoxicity induced by *Cerastes cerastes* venoms were significantly inhibited by the mixture of different amounts of E1 and E2 and the venom. However, both extracts showed neutralization of the venoms hemolytic activity which is more important with E2. Chemical characterization of E1 and E2 revealed there is an important amount of polyphenols and tannins which are known by their bio-active effect. The result could lead as to think that these components have an action by inhibiting the toxicity of snake venoms *in vitro*. We can conclude therefore that the use of the essential oil from the two *Artemisia* species have a curative effect on the snake venom. These results confirmed the traditional use of *A. campestris* and we discovered a similar effect of *A. herba-alba*. No data have been reported till now about the essential oil curative effect of both species.

Keywords: Snake venom; Artemisia; Essential oils; cytotoxicity; lethality

Abbreviation: BPB: Bromophenol blue; *C. c*: *Cerastes cerastes* venom; E1: *Artemesia campestris* essential oil; E2: *Artemesia herba-alba* essential oil; GC–MS: Gas chromatography-mass spectrometry; HMEC-1 cells: Human Microvascular Endothelial Cell; LD_{50}: lethal dosage value; MiHD: The minimum indirect hemolytic dose; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; OPD: o-phenylenediamine; SDS: sodium dodecyl sulfate.

Introduction

The medicinal plant gained more importance as source of bioactive compounds to defeat many diseases. Some substances showed an antitumor effect like Artemisinin, an active constituent of Chinese wormwood (*Artemisia absinthium*) which is a potent antimalarial and recently research showed its antiangiogenic properties (Efferth *et al*., 2001; 2002). Methanol Stem Bark Extract of *Brachystegia eurycoma* possesses significant anti-inflammatory and analgesic properties (Igbe *et al*., 2012).

The phytotherapy offers an opportunity to reconsider the treatment of snake bites, especially in Africa. The cost relatively high of the antivenom serotherapy makes the recourse to this treatment not easy. In some places in the world different plant species were used in popular medicine for protection against snake bites (Mors *et al*., 2000).

The antiophidian effect of many medicinal plants has been used in the traditional medicine. However, just some species have been studied and still a lot of active compounds are not extracted and identified. In our study we have demonstrated the antivenom protection effect of the essential oils of two Tunisian plants *Artemisia campestris* and *A. herba-alba*. The first species have been used in the folk medicine to protect against snake bite in the semi arid and arid region of Tunisia. This is the first study testing the essential oil extract of the two species against the snake venom.

Materials and Methods

Physico-chemical Analysis of Essential Oil Extracts: Techniques and Analytics Methodology

Gas chromatography and retention indices: Due to their physico-chemical nature, the essential oil extracts (a mixture

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of volatiles compounds) were adapted very well to an analysis by gas phase.

The gas chromatography (GC) is indeed a technique of separation of gaseous compounds which could be vaporized by heating without decomposition. The result of a GC analysis is recorded as a chromatogram representing a succession of peaks characterized by retention times and areas. These areas depend on the used detector and are generally proportional to the concentrations of the molecules forming the mixture initially injected.

GC identification of compounds is mainly due to their retention time. This approach faces a major limitation related to the fact that this quantity is directly dependent on the nature of the stationary phase used, but the experimental conditions (particularly temperature programming). To overcome these experimental conditions which vary from one analyst to another, and in order to obtain usable quantities as fixed references, it uses what is called "Kovats Indices (KI)" or "retention indices (RI)."

Retention indices (RI) is calculated using as a reference the retention time of a reference range of linear alkanes (n-alkanes) using the following formula:

• n, n + 1: carbon atoms number of the reference compounds
• R (x): retention time of the compound to be analyzed
• T'R (n): reference compound retention times which elutes before X
• R (n + 1): reference compound retention times which elutes after X
• Cn: carbon number in the series of n-alkanes (example: Cn = 8 octane (C8 noted), Cn = 9 nonane (C9 noted)

$$IR = 100 * C_n + 100.(C_{n+i} - C_n)\frac{t_{R(x)} - t_{R(n)}}{t_{R(n+i)} - t_{R(n)}}$$

Note that if the oven programming is isothermal (constant temperature), it is called "Kovats indices" (IK), whereas if operating with a temperature program (temperature gradient), then it is in this case of "retention index" (IR). In both cases, the calculated ratios are then compared with those for the reference product (measured in the laboratory or described in the literature). Note that it is common to observe changes, sometimes significant, when comparing retention indices obtained in the laboratory and those in the literature (in particular polar column) (Adams, 2007).

Contribution of Mass Spectrometry

Given the very large number of compounds commonly found in samples of essential oils (which can lead to co-elution and therefore identical retention indices), this identification criteria must be complemented by other analytical data, such as those provided by the coupling between the GC and mass spectrometry.

The GC-MS [gas chromatography (GC) with mass spectrometry (MS)] gives indeed a very important possibility of identification of volatile compounds from a mixture. A mass detector placed at the column outl*et al*lows the production of mass spectra for each of the eluted components. These spectra are then compared with those of the reference products in commercial computerized library containing several thousands of spectra (McLafferty, 1989).

Both identification criteria proposed (namely "the mass spectrum" in addition to "confirmation with the retention indices") are generally sufficient to ensure with a great probability the identity of the test compound. However, the current accredited laboratories requires the use of two retention indices for each compound, calculated respectively at two different stationary phases (polar and apolar). Unfortunately, this recommendation has not been followed as part of this work, availability of polar column fault.

Operating Conditions

Analyses were performed using an Agilent brand chromatograph (6890N model) equipped with an auto sampler (Agilent 7683B and coupled to a mass detector (Agilent 5973 MSD). The data were treated at the Using the software "ChemStation" and "Nist Search", armed with mass spectral libraries other experimental conditions are described in the following:

- Capillary column HP-5MS (30 m * 0.25 mm * 0.25 µm) (column length * inside diameter * film thickness of the stationary phase).
- Injection method: Injection with division (split) in a ratio of 1: 200. The temperature of the injector was set at 250°C and the volume injected is 0.2 µL.
- Program temperature: Initial temperature set at 40°C (hold for 1 min) and the temperature rise to 300°C with a level of 4°C/min. The final temperature (300°C) is finally held for 10 min. The total time of the analysis is 76 min.

The composition of *A. campestris* E1 is depicted in Table 1 while that of *A. herba-alba* E2 is given in Table 2.

Venom collection

The Venom collection is realized from two species of snake: *Cerastes cerastes and Macrovipera lebetina* at the Pasteur Institute's serpentarium (Tunis, Tunisia) then stored at -20°C. Human fibrinogen and Rat type IV Collagen were purchased from Sigma Chemical (St. Louis, USA). Cell culture supplements and chemical product were obtained from GIBCO (Cergy-Pontoise, France).

Essential oil Extracts and Proteins Association

To verify the association between the extracts and proteins, SDS-PAGE at 12% was performed according to Laemmli (Laemmli, 1970) with 50.0 µg of *C. cerastes* venom (*C. c*) and mixed with 1000 µg of each extract E1 (*A. campestris* essential oil) E2 (*A. herba-alba* essential oil).

Table 1: Chemical composition (%) of *A. campestris* essential oil

N°	RT (min.)	Compounds	CAS	IR_{Lit}	IR_{cal}	% Total peak area
1	9,05	α-Thujene	2867-05-2	925	917	0,32
2	9,30	α-Pinene	80-56-8	931	925	11,83
3	9,77	Camphene	79-92-5	949	939	0,17
4	9,97	Verbenene	36262-09-6	951	946	0,06
5	10,85	β-Pinene	127-91-3	965	973	33,7
6	11,34	β-Myrcene	123-35-3	992	988	4,35
7	11,49	Cyclooctene oxide	286-62-4	-	993	0,14
8	11,77	α-Phellandrene	99-83-2	1003	1002	0,07
9	12,22	α-Terpinene	99-86-5	1019	1014	0,67
10	12,55	*O*-Cymene	527-84-4	1022	1023	6,73
11	12,71	Limonene	138-86-3	1033	1028	10,19
12	13,03	β-cis-Ocimene	3338-55-4	1037	1036	0,87
13	13,41	β-trans-Ocimene	3779-61-1	1045	1047	1,21
14	13,80	γ-Terpinene	99-85-4	1061	1057	3,18
15	14,85	Terpinolene	586-62-9	1086	1086	0,57
16	15,31	β-Linalool	78-70-6	1097	1099	0,42
17	15,50	β-Thujone	471-15-8	1116	1104	0,51
18	15,79	Fenchol	1632-73-1	1111	1112	0,07
19	15,90	α-Thujone	546-80-5	1112	1115	0,22
20	16,07	Trans-2-menthenol	29803-81-4	1120	1120	0,23
21	16,25	α-Campholenal	4501-58-0	1127	1125	0,18
22	16,70	Trans-pinocarveol	547-61-5	1140	1137	0,85
23	16,90	Camphor	464-48-2	1139	1143	0,3
24	17,08	2,6-nonadienol	28069-72-9	1160	1147	0,2
25	17,56	Pinocarvone	30460-92-5	1168	1161	0,38
26	17,71	Borneol	507-70-0	1165	1165	0,07
27	17,75	α-Phellandren-8-ol	1686-20-0	1165	1166	0,12
28	17,98	Umbellulone	24545-81-1	1170	1172	0,07
29	18,13	(−)-Terpinen-4-ol	20126-76-5	1181	1176	3,23
30	18,43	Crypton	500-02-7	1186	1185	0,33
31	18,60	α-Terpineol	98-55-5	1196	1189	1,09
32	18,80	Myrtenal	564-94-3	1194	1195	0,82
33	19,20	Trans-piperitol	16721-39-4	1205	1206	0,15
34	19,60	Cis-carveol	1197-06-4	1225	1218	0,17
35	20,16	Cis-3-hexenyl isovalerate	35154-45-1	1238	1235	0,41
36	20,35	N-hexyl iso-valerate	10032-13-0	1242	1240	0,19
37	20,45	Carvone	99-49-0	1244	1243	0,18
38	20,83	Geranyl vinyl ether	-	1250	1254	0,31
39	21,53	Phellandral	21391-98-0	1276	1275	0,11
40	24,89	α-Ylangene	14912-44-8	1374	1375	0,13
41	25,10	Geraniol acetate	105-87-3	1380	1381	1,54
42	26,30	Caryophyllene	87-44-5	1422	1419	0,11
43	27,36	α-Caryophyllene	6753-98-6	1453	1454	0,09
44	28,06	γ-Muurolene	30021-74-0	1477	1477	0,25
45	28,21	Germacrene D	23986-74-5	1484	1482	0,94
46	28,68	Germacrene B	15423-57-1	1494	1497	0,14
47	28,78	α-Muurolene	31983-22-9	1499	1501	0,12
48	28,98	α-Farnesene	502-61-4	1505	1507	0,17
49	29,20	γ-Cadinene	39029-41-9	1513	1515	0,14
50	29,47	δ-Cadinene	483-76-1	1524	1524	0,48
51	30,63	Nerodlidol	7212-44-4	1561	1562	0,24
52	31,12	Spathulenol	6750-60-3	1585	1578	2,14
53	31,28	Caryophyllene oxide	1139-30-6	1583	1583	0,35
54	31,42	β-Cedren-9-α-ol	-	1586	1588	0,08
55	31,91	Geranyl isovalerate	109-20-6	1613	1604	1,77
56	32,29	τ-Cadinaol	5937-11-1	1618	1619	0,09
57	32,59	γ-Eudesmol	1209-71-8	1631	1630	0,42
58	32,94	τ-Muurolol	19912-62-0	1648	1642	0,55
59	33,21	β-Eudesmol	473-15-4	1651	1652	2,96
60	33,28	α-Cadinol	481-34-5	1663	1655	0,47
61	34,17	Eudesma-4,11-dien-2-ol	-	1691	1687	0,27

Table 2: Chemical composition (%) of *A. herba-alba* essential oil

N°	RT (min.)	Compounds	CAS	IR_{Lit}	IR_{cal}	% Total peak area
1	9,04	α-Pinene	80-56-8	931	917	0,03
2	9,77	Camphene	79-92-5	949	940	0,78
3	10,66	Sabinene	3387-41-5	972	968	0,24
4	10,76	β-Pinene	127-91-3	972	971	0,08
5	12,54	*O*-cymene	527-84-4	1022	1023	0,55
6	12,80	Cineole	470-82-6	1030	1031	3,73
7	15,82	Thujone	546-80-5	1112	1113	22,88
8	16,16	Chrysanthone	1125-12-8	1124	1123	8,15
9	16,39	Chrysanthenone	473-06-3	1123	1129	2,59
10	17,12	Cis-sabinol	471-16-9	1140	1149	8,90
11	17,20	Camphor	76-22-2	1146	1151	6,02
12	17,50	Sabina ketone	513-20-2	1158	1159	0,17
13	17,67	Pinocarvone	30460-92-5	1168	1164	0,67
14	17,84	Borneol	507-70-0	1165	1169	1,81
15	18,20	(−)-Terpinen-4-ol	20126-76-5	1181	1179	0,59
16	18,42	α-Thujenal	?	1182	1185	0,13
17	18,65	α-Terpineol	98-55-5	1196	1191	0,14
18	18,82	Cis-piperitol	16721-38-3	1199	1196	0,31
19	19,26	Trans-piperitol	16721-39-4	1205	1208	0,49
20	20,35	Cumaldehyde	122-03-2	1236	1241	0,21
21	20,49	Carvone	99-49-0	1244	1245	0,10
22	20,86	Piperitone	89-81-6	1258	1256	0,33
23	21,92	L-Bornyl acetate	5655-61-8	1284	1287	0,14
24	22,48	Myrtenyl acetate	1079-01-2	1306	1304	35,53
25	22,58	Carvacrol	499-75-2	1311	1307	0,14
26	23,16	2,6-dimethyl-3,5-Heptadien-2-ol	77411-76-8	?	1324	0,14
27	25,67	Jasmone	488-10-8	1393	1398	0,37
28	28,24	Germacrene D	23986-74-5	1484	1483	0,09
29	28,71	Bicyclogermacrene	67650-90-2	1495	1499	0,07
30	29,16	Davana ether	35470-57-6	1491	1514	0,19
31	31,15	Spathulenol	6750-60-3	1585	1579	0,58
32	31,30	Caryophyllene oxide	1139-30-6	1583	1584	0,11
33	32,22	Vinyl cyclohexanecarboxylate	4840-76-0	?	1616	0,17
34	34,06	Cis-6-dodecen-4-olide	18679-18-0	1675	1684	0,10

In Vitro Determination of Essential oil Effect on Fibrinogen Hydrolytic Activity

Extracts E1 and E2 were added separately to a solution of 0.1% human fibrinogen mixed with 50 mM of Tris-HCl buffer (at pH 7.5) (a volume of 1 mL) and a venom solution concentration of (20 µg) we used as reference the method of Ouyang and Teng (1976). The mixture was incubated in the presence at first and then in absence of extracts (1 mg) at 37°C. 100 µL of denaturing solution aliquots was added (10 mM phosphate buffer, pH 7.2, containing 10 M urea, 4% sodium dodecyl sulfate (SDS), and 4% β-mercaptoethanol) at various time intervals. Then the solution was incubated at 37°C for 6 h and run on 10% polyacrylamide slab gel electrophoresis which was carried out for 2 h with a current of 25 mA per slab gel. The Bromophenol blue (BPB) solution was used as an indicator.

In Vitro Determination of the Essential Oils Effect on Caseinolytic Activity

We used a modified protocol of Rodrigues *et al*. (2000). In this method we choose casein as substrate with 40 µg of *C. cerastes* crude venom. The same assay was carried out adding the essential oils E1 and E2 separately after pre-incubation for 30 min at 37ºC with different venom concentrations (w/w). To define one unit of caseinolytic activity we consider the increase in absorbance of 0.001 U/min produced by the amount of venom/protease.

In Vitro Determination of the Essential Oils Effect on Collagen Hydrolytic Activity

The test was realized using the following method: Sodium hydrogen carbonate solution (60 µL, pH 12) was added to 0.3% type IV collagen (0.9 mL) and adjusted to pH 8. 20 µg of *C. cerastes* were incubated with type IV collagen aliquots in the presence at first then in absence of Extracts E1 and E2 (1 mg). Aliquots of 100 µL of denaturing solution (10 mM phosphate buffer, pH 7.2, containing 10 M urea, 4% SDS, and 4% β-mercaptoethanol) were added during various interval time. This solution was boiled for 3 min and run on SDS-PAGE using a 7.5% polyacrylamide slab gel electrophoresis.

In Vitro Determination of Essential Oils Effect on L-Amino Acid Oxidase Activity

We used Kishimoto and Takahashi (2001) method. *C. cerastes* venom or LAAO was pre-incubated with extracts

for 30 min at 37°C for the inhibition test.

Essential Oils Effect on Phospholipase Activity A2

A_2 activity was measured based on its indirect hemolytic action on agarose gel, using egg yolk and red blood cells as substrate. The minimum indirect hemolytic dose (MiHD) was defined as being the amount of venom that could produce a 10 mm diameter of hemolytic halo. To evaluate the ability of Extracts to inhibit phospholipase activity we pre-incubated each extract E1 and E2 with MiHD of *C. cerastes* or *M. lebetina* venoms (1:10 w/w). Control samples contained venom without Plant extracts. Plates were incubated at 37°C overnight. The efficacy of the two species essential oils in neutralizing the phospholipase activity was expressed as 100% inhibition corresponding to the absence of a hemolytic halo. Each assay was performed in triplicate and expressed as mean ± standard deviation of the mean.

Essential Oils Effect on Venom Cytotoxicity on HMEC

Cell culture: HMEC-1 cells have been cultivated according to Bourdron *et al*. (2006).

Essential Oils Effect on Venom Cytotoxic Action

Cerastes cerastes venom on HMEC-1 cells was investigated. The cells were trypsinized at first then washed with the same medium and re-suspended in the growth media. These cells were seeded in 96-multiwell plates (5×10^3 cells per well in 100 µL medium) till they attach and reach log phase of growth. Various concentrations of essential oils E1 and E2 (µg/mL in 10% DMSO-saline) in the presence or absence of *C. cerastes* venom were added to each well in 100 µL medium. Cell viability was tested by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) according to Mosmann (1983) protocol. We used spectra Max microplate reader (Thermo scientific, USA) for the absorbance at 550 nm.

***In Vivo* Test**

Acute toxicity of the extracts and venom: The venom lethality was tested by subcutaneous routes in albino mice (18–22 g). Various concentrations of *C. cerastes* and *M. lebetina* venoms solution (venom dissolved in saline solution) were used (1.0–10 mg/mL). We have injected 100 µL of each solution. For control mice received saline solution without adding venom. Deaths were recorded during 48 h and LD_{50} was calculated by probits.

The effect of extracts on the neutralization of toxicity of snake venom was determined by mixing each extract with snake venom ($2LD_{50}$) before injection. Deaths were registered within 24 h and results were the mean.

Edema-inducing activity: Intradermal injection of 20 µg from *C. cerastes* venom was realized in the right foot pad of male albino mice (weight: 18–22 g) to induce edema. To verify the essential oil inhibition action on venom, we incubated the venom with different concentration of E1 and E2 (w/w) separately. 50 µL of phosphate-buffered saline (PBS, pH7.2), DMSO or plant extracts were injected to the control groups. To determine the edema progression a low pressure pachymeter (Mitutoyo, Japan) have been used after 24 h.

Results

Essential Oil Composition

The major compounds of the *A. campestris* essential oil were the β-pinene (33.7%) followed by the α-pinene and Limonene (11.83%, 10.19%) (Table 1). For *A. herba-alba* essential oil the major compounds were Myrtenyl acetate (35.53 %) followed by thujone (22.88%) (Table 3).

***In Vitro* Test**

Interaction between snake venoms and essential oils (SDS-PAGE): We have noted absence of visible change in the electrophoretic pattern of *C. cerastes* venom, during its incubation with extracts (Fig. 1). This is eliminating the hypothesis of the protein degradation as a possible reaction. Furthermore, no proteins in the extracts were detected.

Fibrinogen hydrolytic activity assay: The human fibrinogen incubation with *C. cerastes* venom (*C. c*) revealed that the Aα band of the fibrinogen disappeared on SDS-PAGE, whereas the β chain and γ chain were unaffected (Fig. 2a). The mixture of venom and plant extracts (E1 and E2) did not show a significant protection of the degradation of human fibrinogen (Fig. 2b and c).

Collagen hydrolytic activity assay: Type IV collagen was incubated with *C. cerastes* venom at different periods of time. The venom degraded completely type IV collagen (104 kDa), especially over 1 h, to smaller molecular weights (43 and 35 kDa)(data not shown). In the presence of extracts (0.5 mg/mL), type IV collagen was digested by incubation with the venom (data not shown).

Proteolytic activity on casein: The E1 showed more important caseinolytic hydrolytic activity inhibition beginning with the ratio of 1:10 (Fig. 3a), while the E2 has less inhibition effect for the same rate (Fig. 3b).

LAAO activity assay: The LAAO activity test showed no significant decrease of the enzymatic activity of crude venom with the presence of E1 or E2 (Fig. 4a). Furthermore, purified L-amino acid oxidase of *C. cerastes* venom was not inhibited by any of the extracts (E1 and E2) (Fig. 4b).

Table 3: Neutralization of the indirect hemolytic activity of *C. cerastes and M. lebetina* venoms by extracts of plants

Venom	Plant extract	% of inhibition
CC	E1	31% ± 5
	E2	49% ± 3
MVL	E1	27% ± 2
	E2	34% ± 3

Both extracts were tested against one minimum indirect hemolytic dose (MiHD) (5 µg) of CC or MVL venoms, in agarose-erythrocyte-egg yolk gels. Experiments performed by triplicate

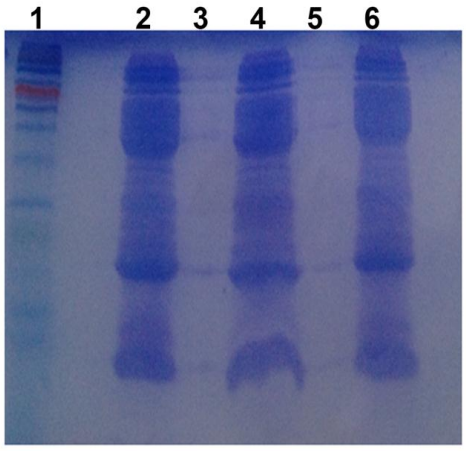

Fig. 1: Electrophoretic analysis of the interaction between venom and extracts on SDS-PAGE. Lane 1- molecular mass markers; lane 2- *C. cerastes* crude venom; lane 3- E1, lane 4- E1 and crude venom, lane 5- E2 and lane 6- E2 and crude venom

Indirect haemolytic assay: The two extracts demonstrated neutralizing activity against C.C and MVL venom which is more significant with E2 (Table 3).

Inhibition of Venom Cytotoxic Action on HMEC

E1 or E2 alone had no effect on the viability of HMEC. The cell viability is more important when the E1 and E2 were added and it significantly protected HMEC against cytotoxic effects of *C.C* venom at all concentrations of the tested extracts. The maximum (99%) protective effect was exhibited with E1 at 2.5 µM (Fig. 5a) than E2 with 95% (Fig. 5b). The anti-venom effect is much higher for E1 than E2 after incubation and it is similar for both extract without prior incubation (Fig. 5c).

***In Vivo* Tests**

Essential oils neutralization effect on venom lethality: Subcutaneous viper venom ($2LD_{50}$) has been injected into male albino mice than an injection of E1 and E2 has been injected later (1 mg per mouse PO). The *C.C and VL* venom lethality has been remarquebly stopped by E1 and E2. The first extract added to the *C.C* venom showed one survival

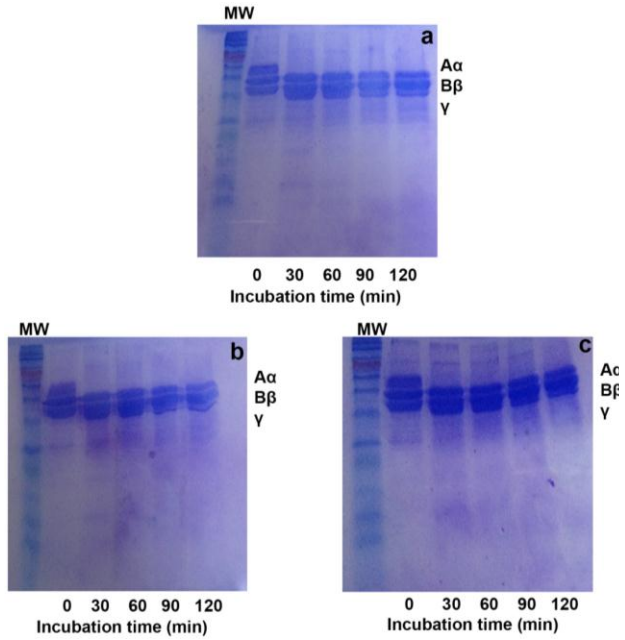

Fig. 2: Effects of Extracts on human fibrinogen hydrolytic activities by *C. cerastes* venom. 10% SDS-PAGE of time-dependent digestion of human fibrinogen by *C. cerastes* venom in the presence or absence of Extracts. (a) *C. cerastes* venom without Extracts; (b) *C. cerastes* venom with E1; (c) *C. cerastes* venom with E2

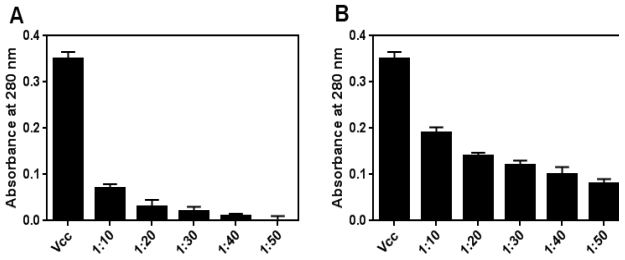

Fig. 3: Effects of Extracts on proteolytic activity on casein. (a) *C. cerastes* venom with E1; (b) *C. cerastes* venom with E2

mouse and the second extract 3 survival while the effect of the two extracts added to VL is less curative since for the first extract all mice were dead and for the second extract just one mouse survived.

Anti-inflammatory activity: The injection of 0.01 mL venom was followed by a remarquebale inflammation reaction in mice paw (at 1 h), while the Pre-treatment with *E1* and *E2* (1 mg each) per mouse, PO before the injection of *C.C* venom reduces this reaction. The minimization of oedema reaction was found to be almost similar for both extracts. In our study we realized that the essential oils E1 and E2 neutralized significantly the inflammation effect of the venom and therefore the oedema (Fig. 6).

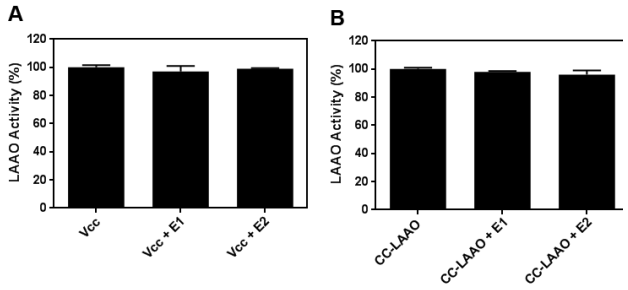

Fig. 4: Effects of Extracts on LAAO activity. (a) *C. cerastes* venom with Extracts; (**b**) Purified LAAO with Extracts

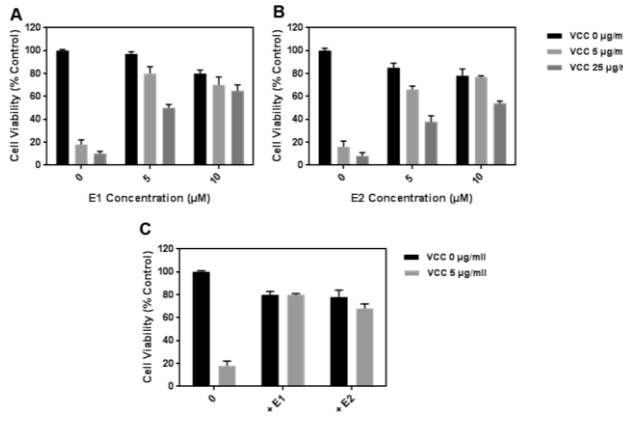

Fig. 5: Effect of Extracts against the cytotoxic actions of *C. cerastes* venom on HMEC. (a) Effect of E1, (b) Effect of E2, (c) Effect of Extracts against the cytotoxic actions of *C. cerastes* venom on HMEC without pre-incubation

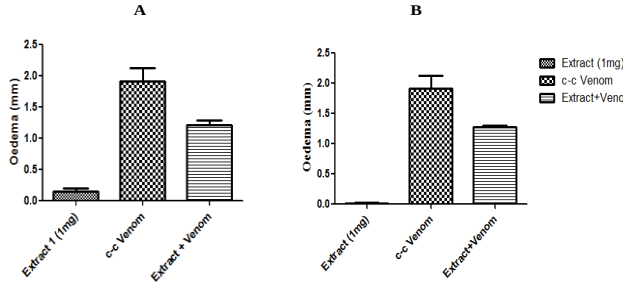

Fig. 6: Anti-inflammatory activity of extracts

Discussion

The envenomation by snakes causes generally local tissue damage, consisting of oedema, haemorrhage, myonecrosis, haemolyse and inflammation. Many plant families have been used in traditional medicine as anti ophidian by treating damage caused by the snake venom (Andreimar *et al*., 2005). Studies focused in some species to investigate and isolate the bioactive compounds from different part of the plant. The extracts from different parts of the plants belonging to the Asteraceae family showed an anti-hemorrhagic, antiproteolytic, anti-phospholipase (Abid *et al*, 2007; Mariane *et al*, 2011), antilethality (Shirwaikar *et al*., 2004; Pithayanukul *et al*., 2009), antiedema, (Ticli *et al*., 2005; Nishijima *et al*., 2009), antinecrosis (Bjarnason *et al*., 1978; Houghton *et al*., 1992; Chareanchai *et al*., 2009; Soares *et al*., 2005), anti-myotoxicity (Theakston and Reid, 1992; Bjarnason and Fox, 1994; Soares *et al*., 2004) and anticoagulant effect (Bjarnason and Tu, 1978; Bjarnason and Fox, 1994). Our results have some similarities with those research investigations. The use of the Artemisia essential oils extract reduced the lethal effect on the mice. The extract has a neutralizing capacity against C.C venom. The same effect has been noticed with 74 ethanolic extracts of plants used in the folk medicine in Colombia (Otero *et al*., 2000). The essential oils of the two *Artemisia* species were composed of an important amount of phenolic compounds. It has been demonstrated that many phenolic compounds have an antivenom action such ar-turmerone extracted from *Curcuma longa* roots, the compound have been shown to neutralize the hemorrhagic and lethal effect induced by some venoms (Dob and Benabdelkader, 2006), tannic acid has an hemorrhagic neutralization effect (Maugini, 1930), 2-hydroxy-4-methoxy benzoic acid isolated from the methanolic root extract of *Hemidesmus indicus* neutralyzed inflammation (Ipshita and Chakravarty, 2006). According to Sanchez and Rodríguez (2008) the polyphenols can bind proteins and make the venom unable to act on receptors and act by competitive blocking of the receptors. These tests can explain the neutralized effect of *A. campestris* and *A. herba-alba* essential oils against the venom. Both species showed a decrease of the lethality effect of C.C venom and its cytotoxicity on HMEC. It has been shown that the two essential oils have an anti-haemolytic effect like the most of the Asteraceae species extracts. The anti-venom actions are positive when the venom is mixed with the essential oils before the biological tests.

In this study we confirmed the biological anti-venom effect of *A. campestris* already used in the traditional medicine and we demonstrated a similar effect of *A. herba-alba*. Further studies will be performed to identify and isolate the pure compounds responsible for the venom inhibition from the two essential oils.

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