Isolation and Identification of Anti-Ulcer Components from *Anchusa Strigosa* Root

Manal Abbas[1]✉, Ahmad Disi[1], Suleiman Al-Khalil[2]

[1]Faculty of Science, the University of Jordan, Amman, Jordan.
[2]Faculty of Pharmacy, the University of Jordan, Amman, Jordan.

ABSTRACT

Anti- ulcer activity of different root extracts of *Anchusa strigosa* was studied in ethanol-induced ulcer model in rats. Petroleum ether-soluble fraction was the most effective in reducing ulcer index and gave 91% protection. Chloroform-soluble fraction gave 86% protection while butanol-soluble fraction was less effective (65% protection). On the other hand, water-soluble fraction was not effective in protecting the stomach from the ulcerative agent. Phytochemical analysis of petroleum ether fraction resulted in the isolation of four compounds. Identification of these compounds depended on melting point, U.V, I.R, H and C-13 NMR and MS analysis. These compounds were identified as: Oleanolic acid, Beta-amyrin, Crataegolic acid and Beta-sitosteryl glucoside. These triterpenes were isolated from *A. strigosa* roots for the first time. Alkaloids were detected in roots of Anchusa strigosa which may put the use of this plant in folk medicine in question.

Keywords: *Anchusa strigosa*, Anti-ulcer action, triterpenes, Oleanolic acid, Beta-amyrin, Crataegolic acid, Beta-sitosteryl glucoside..

INTRODUCTION

Anchusa strigosa Banks et Sol. (Boraginaceae) is a perennial herb that is very common and widely distributed in Mediterranean and Irano-Turanian biotopes in Jordan(1). It is used locally and in neighboring countries as antiulcer(2), for wound healing(3), as a tonic and tranquilizer(4), and as a diuretic and for abdominal pain(5). Abuereish(6) showed that the aqueous root extract of *A. strigosa* inhibited pepsin activity in vitro. Disi et al.(2) studied the antiulcer activity of the aqueous root extract of *A. strigosa* in rats after inducing peptic ulcers for them using absolute ethanol. However, no previous studies have isolated active ingredients from this plant. So, this study aims at: 1.Fractionation of the root of *A. strigosa* using different solvents.

Received on 25/8/2008 and Accepted for Publication on 11/1/2009.
✉ E-mail: manabbas@yahoo.com

2. Determine which fraction of the root extract of *A. strigosa* has anti-ulcer activity in rats. 3. Purification and identification of chemical constituents of the active fractions of the root. 4. Investigating the presence of alkaloids in roots of this plant.

MATERIALS AND METHODS:

Collection of plant material: *Anchusa strigosa* was collected from Jordan University campus, Amman, Jordan in March and April. The plant was authenticated by the Department of Biological Sciences, The University of Jordan. A voucher specimen was deposited at the Herbarium/Department of Biological Sciences/The University of Jordan.

Anti –ulcer action: Albino rats (*Rattus norvegicus*) of both sexes weighing 200-250 grams were used. Ethanol-induced ulceration was produced according to Otani(7) with slight modifications: Rats housed in wire-floor cages (to prevent coprophagy) were fasted for 36

hours. Ten percent sucrose was available ad libitum instead of water, but *removed* one hour before the animals received the first treatment. Animals were divided randomly into five groups (six animals each). Animals in each group received one of the following treatments by oral intubation: Distilled water (control group), petroleum ether-soluble fraction, chloroform-soluble fraction, butanol-soluble fraction, or water-soluble fraction. These fractions were obtained as follows: Fifty grams of 70% ethanolic extract of powdered roots of *A. strigosa* were dissolved in 1L distilled water and partitioned sequentially with equal volume of petroleum ether, chloroform and butanol (three times each). The obtained fractions as well as the solution that remained after sequential partitioning (aqueous-soluble fraction), each fraction was dried under reduced pressure and then dissolved or suspended in distilled water to obtain a concentration of 5g/100 ml. Each faction was boiled for fifteen minutes in order to mimic the preparation method used in folk medicine and its concentration was readjusted to be 5g/100 ml. After cooling to room temperature, the freshly prepared fractions were administered to animals in different groups via oral intubation (5 ml/kg). After 90 minutes, absolute ethanol (5ml/kg) was administered by oral intubation to all animals. Sixty minutes later, animals were sacrificed with ether over dose. After dissection, the stomach was removed, opened along the lesser curvature, gently washed with normal saline, spread and photographed. Total lesion area in the stomach as well as total area of the glandular portion were measured using digital planimeter (Pla COM K-90) following Konturek et al.[8]. Ulcer index was obtained by dividing the total area of the lesions in the stomach by the area of the glandular portion of stomach. Protection ratio of each fraction was calculated using the formula:

$$\text{Protection ratio} = 100 - \frac{\text{Ulcer index(test)}}{\text{Ulcer index(control)}} \times 100$$

Phytochemical analysis: Eight Kg of finely powdered, air dried roots of *A. strigosa* were extracted with 70% ethanol containing EDTA at room temperature five times; seven days each. Ethanol was evaporated at reduced pressure to leave a crude gummy material (325 gm) ethanolic extract of powdered roots of *A. strigosa* was dissolved in distilled water and partitioned sequentially with equal volume of petroleum ether, chloroform and butanol (three times each). The obtained fractions as well as the solution that remained after sequential partitioning (aqueous-soluble fraction) were dried under reduced pressure. Petroleum ether soluble fraction (30.5 gm) was applied to a 100 cm x 2.6 cm column containing 130 gm silica gel 60H. Chloroform extract(21.7 gm) and butanol extract(1.3 gm) were combined and applied to another column (100cm x 3.4 cm) filled with 100 gm of the same packaging material. The polarity was gradually increased by the addition of chloroform and chloroform-methanol mixtures to afford various fractions which were collected and dried under reduced pressure. Thin layer chromatography (TLC) was done using 20x 20 cm glass plates coated with silica gel. Different solvent systems were used such as: chloroform:ethylacetate (1:1), chloroform: petroleum ether (1:1) and ethylacetate. The spraying reagent used to develop TLC chromatograms was 95% ethanol-H_2SO_4-anisaldehyde (18:1:1). Purification of compounds from fractions obtained by column chromatography depended on recrystalization from organic solvents (e.g methanol, acetone,…) or performing TLC on preparative TLC plates prepared at our laboratory by spreading silica gel 60H dissolved in distilled water and well mixed over a 20x20 cm glass plates. The prepared plates were left at room temperature to the next day in order to solidify and then heated in oven at 100ºC for 9 hours.

Identification of purified compounds: Identification of pure compounds depended on the following tests:

1. Melting point determination: using electrothermal Mel- Temp apparatus.
2. Ultraviolet spectral analysis: using Perkin-Elmer model 552 A UV/VIS spectrophotometer with a model 561 recorder.
3. Infra spectral analysis: infrared spectra were taken in KBr pellets using a Perkin-Elmer model 267

grating infrared recording spectrophotometer.

4. Mass spectrometric analysis: low resolution mass spectra were recorded on Varian MAT 311 instrument.

5. Nuclear magnetic resonance spectrometric analysis: the proton and carbon 13 nuclear magnetic resonance (NMR) spectra were taken at 400 MHz on a Bruker WP 89 SY spectrometer.

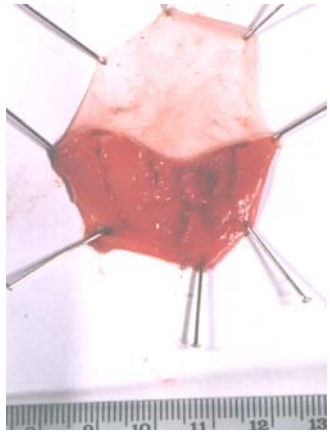
Extraction of alkaloids: One hundred grams of 70% ethanolic extract of the roots were dissolved in one liter distilled water and acidified with 5% tartaric acid(final pH =2.5). After filtration, the aqueous solution was partitioned with diethyl ether (1L). The aqueous layer was basified with ammonia (final pH=9). Then, it was partitioned with chloroform. The chloroform layer was dried under reduced pressure and subjected to thin layer chromatography analysis to check the presence of alkaloids in this layer. Dragendorff's reagent was used as spraying agent.

RESULTS

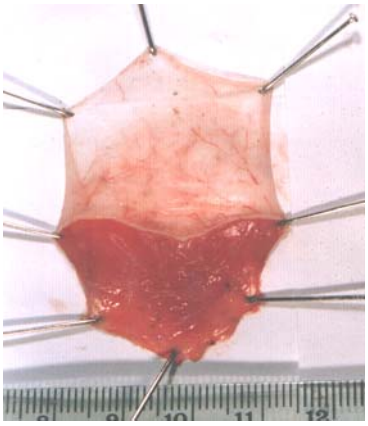
Anti-ulcer action: A significant difference in ulcer index between different groups was observed ($p<0.025$). Mean ulcer index was as follows: Petroleum ether-soluble fraction 0.8% $\pm$0.9, Chloroform-soluble fraction 1.3% $\pm$1.3 , butanol-soluble fraction 3.3%$\pm$3, water-soluble fraction 14.05%$\pm$13.7 and 9.4%$\pm$5.1 for control group. Protection ratio was as follows: Petroleum ether-soluble 91% protection. Chloroform-soluble fraction gave 86% protection while butanol-soluble fraction was less effective (65% protection). On the other hand, water-soluble fraction was not effective in protecting the stomach from the ulcerative agent (Figure 1a-e).

Table (1): ^{13}C-NMR chemical shifts of Oleanolic acid , Beta-amyrin, Crataegolic acid and Beta-sitosteryl glucoside

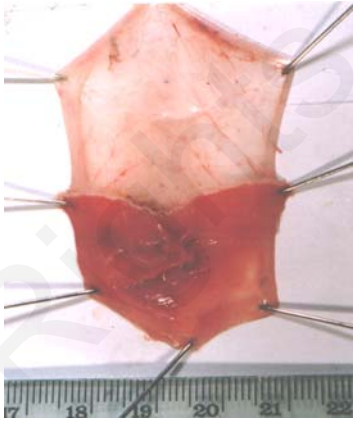
Compound / Carbon #	Oleanolic acid	Beta-amyrin	Crategolic acid	Beta-sitosteryl glucoside
C-1	37.9	37.3	38.5	36.75
C-2	27.2	28.28	66.27	23.96
C-3	76.7	71.85	79.38	76.8
C-4	38.2	37.30	37.30	39.10
C-5	54.7	36.81	47.80	140.33
C-6	17.9	21.12	18.86	121.1
C-7	45.9	42.35	42.64	31.28
C-8	40.87	45.90	45.90	31.31
C-9	47.00	50.18	45.30	49.50
C-10	36.49	36.55	33.70	36.11
C-11	22.8	24.33	22.30	19.94
C-12	120.80	121.70	128.67	38.20
C-13	144.30	140.81	139.60	41.75
C-14	44.19	44.36	44.36	56.08
C-15	26.80	26.13	26.34	25.30
C-16	22.70	23.11	24.30	27.71
C-17	48.48	48.48	54.40	55.32
C-18	40.90	45.89	42.58	11.56
C-19	33.45	39.82	33.73	19.61
C-20	36.49	36.49	36.49	35.40
C-21	33.30	34.00	29.20	18.82
C-22	32.10	31.71	27.00	33.25
C-23	16.80	29.3	17.37	22.50
C-24	15.80	19.84	16.80	45.00
C-25	15.00	19.10	21.60	29.00
C-26	17.70	18.90	18.50	19.00
C-27	25.70	18.30	18.10	20.10
C-28	179.00	19.20	179.0	28.00
C-29	32.80	36.18	29.17	11.62
C-30	27.70	19.42	26.34	
Glucose				
C-1`				10.7
C-2`				73.3
C-3`				76.65
C-4`				69.95
C-5`				76.65
C-6`				60.97



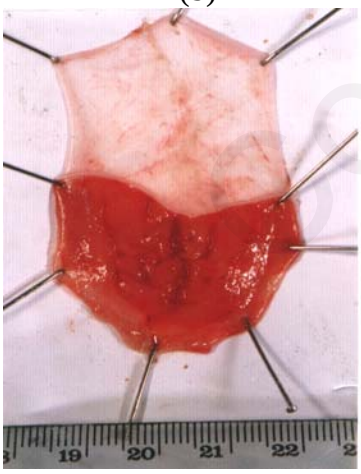
(a)



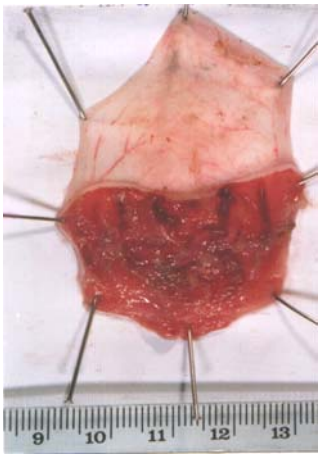
(b)



(c)



(d)

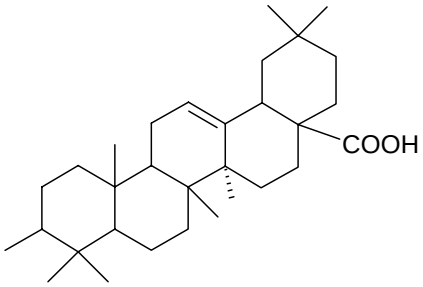


(e)

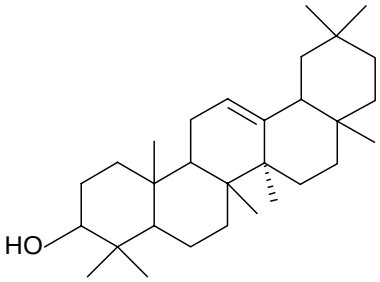
Figure (1): Rat stomach after the administration of different fractions of *A. strigosa* followed by absolute ethanol after 90 minutes a. distilled water (control) b. petroleum ether fraction c. chloroform fraction d. butanol fraction e.

water soluble fraction

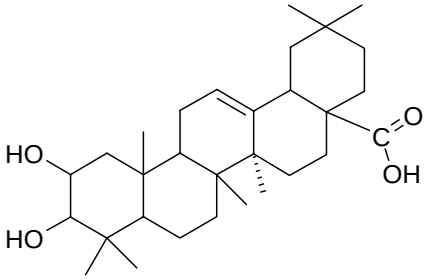
Phytochemical analysis: Four compounds: Oleanolic acid (1), Beta-amyrin(2), Crataegolic acid (3) and Beta-sitosteryl glucoside (4) were purified from petroleum ether fraction. Assignment of the ^{13}C-NMR shifts is shown in table 1.



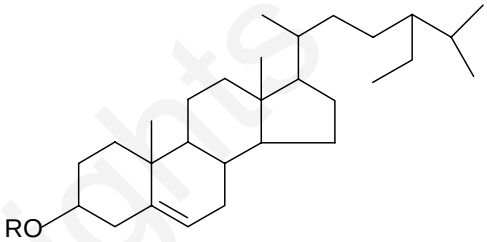
(1)
Oleanolic acid



(2)
Beta-amyrin



(3)
Crataegolic acid



R= β-D- Glucose

(4)
Beta-sitosteryl glucoside

Extraction of alkaloids: Alkaloids were detected in the chloroform layer. Their presence was checked by subjecting the chloroform fractions to thin layer chromatographic analysis. A brown color developed when chromatograms were sprayed with Dragendorff's reagent.

DISCUSSION AND CONCLUSION

Using ethanol- induced ulcer model in rats, our study showed that different fractions of the root were gastroprotective . Petroleum ether fraction of *A. strigosa* root was the most effective (91% protection). This agrees with previous studies that found that the aqueous root extract of *A. strigosa* lowered the ulcer index; obtained by planimetric method from 32.5 % +/- 9.4 to 2.2% +/- 1.4 using the same animal model[(2)]. The purification of oleanolic acid, beta-amyrin and beta-sitosteryl glucoside from petroleum ether fraction of *A. strigosa* may explain; at least in part, the use of this plant in folk medicine for the treatment of gastric ulcer. Recently, many studies proved the anti ulcerogenic effects of these compounds. Navarrete et al.[(9)] reported that a mixture of alpha- amyrin and beta-amyrin from root bark of *Hippocratea excelsa* had 50% gastroprotection in several experimental ulcer models in rats. While sitosterol-3- O- beta- glucoside produced 93.4% protection. Arrieta et al.[(10)] showed that Beta-sitosterol and 3-epi-oleanolic acid from *Amphipterygium adstringens* stem bark have anti-ulcer activity with 88.8% and 42.5% gastroprotection , respectively. Xiao et al.[(11)] found that beta-sitosterol-beta-D-glucoside and its aglycone from *Hippophae rhamnoids* L. seed oil was gastroprotective in two ulcer models in

rats. Both the glucoside and its aglycone showed antiulcerative activity in chronic acetic acid-induced gastric model, and their effects were at least comparable to the effects of wishupin in combination with cimetidine. The effect of the aglycone was better than the glucoside's. Rodriguez et al.[12] reported that oleanolic acid promotes healing of acetic acid-induced chronic gastric lesions in rats. In vitro, oleanolic acid significantly reduced human epithelial gastric cell damage and it increased the prostaglandin content in human epithelial gastric cell cultures[13].

The mechanism of the gastroprotective action of *A. strigosa* root extract is not fully understood. Abuereish[6] showed that *A. strigosa* root extract inhibited pepsin activity in vitro. But further studies are needed to illustrate its mechanism of action in vivo.

This study confirms the presence of alkaloids in *A. strigosa* roots obtained from Jordan. These findings agree with the results of Siciliano et al.[14] and Braca et al.[15] who purified several pyrrolizidine alkaloids from *A. strigosa.* Alkaloid content was highest in leaves and lowest in roots according to Siciliano et al.[14]. The presence of pyrrolizidine alkaloids even at low concentrations in some medicinal plants may limit their use in folk medicine because of their toxicity[14]. Thus, further studies investigating the toxicity of *A. strigosa* in vivo and in vitro are needed.

ACKNOWLEDGEMENTS:

The authors thank Professor Dr. Al-Eissawi for identification of the plant.

REFERENCES

(1) Feinburn-Dothan, N. Flora Palaestina, Part 3. The Israel Academy of Science and Humanities, Jerusalem. 1978.

(2) Disi, A.M., Tamimi, SO, Abuereish, GM. Effects of Anchusa strigosa root aqueous extract on gastric ethanol- induced ulcer in laboratory animals. *Journal of Ethnopharmacology* 1998. 60(3): 189-198.

(3) Dafni, A, Yaniv, Z, and Palvitch, D. Ethnopharmacological survey of medicinal plants in northern Israel. 1984. *Journal of Ethnopharmacology* 10: 295-310.

(4) Al Maliki, S. J., Elisha, E. E., 1985. Effect of some Iraqi medicinal plants on social aggression in albino mice. *Journal of Biological Science Research* 16(2): 249-257.

(5) Al Khalil, S. A survey of plants used in Jordanian traditional medicine. *International Journal of Pharmacognosy* 1995. 33(4): 317-323

(6) Abuereish, GM. Pepsin inhibitor from roots of Anchusa strigosa. *Phytochemistry* 1998. 48(2), 217-221

(7) Otani, K, Yanaura,S, Yuda, Y, Kawaoto, H, Kajita, T, Hirano, F, Osawa, F, Inouye, S. Histo-chemical studies on the anti ulcer effect of bamboo grass in rats. *International Journal of Tissue Reaction* XII(6): 1990. 319-332.

(8) Konturek, SJ, Radecki, J, Brzozowski, J, Drozdowice, D, Piastuccki, I, Muramatsu. M, Tanaka, M, Aihara, H. Antiulcer and gastroprotective effects of solon, a synthetic flavonoid derivative of sophoradin, role of endogenous prostaglandins. *European Journal of Pharmacology* 1986. 125: 185-192

(9) Navarrete, A, Trejo-Miranda, JL, Reyes-Trejo, L. Principles of root bark of Hippocratea excelsa (Hippocrataceae) with gastroprotective activity. *Journal of Ethnopharmacology* 2002. 79(3): 383-388.

(10) Arrieta, J, Benitez, J, Flores, E, Castillo, C, Navarrete, A. Purification of gastroprotective triterpenoids from the stem bark of Amphipterygium adstringens; role of prostaglandins, sulfhydryl, nitric oxide and capsaicin-sensitive neurons. *Planta Medica* 3003. 69(10): 905-909.

(11) Xiao, M, Yang, Z, Jiu, M, You, J, Xiao, R. The antigastroulcerative activity of beta-sitosterol-beta-D-glucoside and its aglycone in rats. *Hua Xi Ke Da Xue Xue Bao* 1992. 23(1): 98-101 (Abstract).

(12) Rodriguez, JA, Astudillo, L, Schmeda-Hirschmann, G. Oleanolic acid promotes healing of acetic acid-induced chronic gastric lesions in rats. *Pharmacology Research* 2003. 48(3): 291-294

(13) Sanchez, M, Theoduloz, C, Schmeda-Hirschmann, G, Razmilic, I, Yanez, T, Rodriguez, J. A. Gastroprotective and ulcer- healing activity of oleanolic acid derivatives: In vitro- in vivo relationships. *Life Sciences* 2006. 79(2006) 1349-1356.

(14) Siciliano, T, Leo, MD, Bader, A, Tommasi, ND, Vrieling, K, Braca, A, Morelli, I. Pyrrolizidine alkaloids from Anchusa strigosa and their antifeedant activity. *Phytochemistry* 2005. 66(13): 1593-1600.

(15) Braca, A, Bader, A, Siciliano, T, Morelli, I, De Tommasi, N. New pyrrolizidine alkaloids and glycosides from Anchusa strigosa. *Planta Medica* 2003. 69(9): 835-841.

عزل مكونات فعالة مضادة للقرحة من جذور نبتة الحمحم

منال عباس[1]، أحمد الديسي[2]، سليمان الخليل[2]

[1] كلية العلوم، الجامعة الأردنية، عمان، الأردن.
[2] كلية الصيدلة، الجامعة الأردنية، عمان، الأردن.

ملخـص

تمت دراسة الأثر المضاد للقرحة للمستخلصات المختلفة لجذر نبات الحمحم (*Anchusa strigosa*) في الجرذان التي تم إحداث القرحة عندها باستخدام الكحول المركز. كان مستخلص البترولیوم ایثر الأكثر فعاليه في تقليل معامل القرحة إذ أعطى حماية بمقدار 91%. أما مستخلص الكلوروفورم فأعطى حماية بنسبة 86% و مستخلص البيوتانول فأعطى حماية بنسبة 65% بينما لم يكن المستخلص المائي فعالا في حماية المعدة. التحاليل الكيميائية للنبات أدت إلى عزل أربعة مركبات تم التعرف عليها عن طريق تحديد طيف الأشعة تحت الحمراء ، طيف الأشعة فوق البنفسجية، طيف الرنين النووي المغناطيسي للهيدروجين و الكربون 13 و طيف الكتلة. ووجد بأنها: حامض الأوليبك، بيتا أميرن، حامض الكرانتجيول و بيتا سيتوستيرول جليكوسايد وهذه المركبات تعزل من الحمحم لأول مرة.

الكلمـات الدالـة: الحمحم، التأثير المضاد للقرحة، التربينات الثلاثية، حامض الاوليبك، بيتا أميرن، حامض الكرانتجيول، بيتا سيتوستيرول جليكوسايد.

تاريخ استلام البحث 2008/8/25 وتاريخ قبوله للنشر 2009/1/11.