Detection of Microscopic Characters And Evaluation Of The Anti-Inflammatory Effect Of Anagallis Arvensis L. Wildly Grown In Karbalaa' Countryside

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Inflammation, Diclofenac, Microscopic, Thrichome, Pollens And Anagallis.

ABSTRACT
Anagallis arvensis is a well-known herb wildly grown in Karbalaa' countryside. The aim of the study was to detect some microscopic features for the dried-grinded roots, leaves, flowers and seeds of the plant and evaluate the effect of the alcoholic extract of the aerials part (AEAP) on acute inflammation induced by egg-white model.
Microscopic identification was done using an optical microscope. For the evaluation of the anti-inflammatory effect, rats were divided into 4 groups (n=6), the negative control group, the positive control (Diclofenac sodium) group, AEAP 4 mg/kg was applied to group 3 individuals and AEAP 7 mg/kg was applied to group 4. The extracts and the positive control were I.P. injected.
The results of the microscopic test revealed several diagnostic characters, such as cluster oxalate crystals and xylem vessels with pitted walls in the roots. Glandular trichomes composed of one- or two-celled stalk and a spherical, unicellular head and Fragments of the fibrous layer of the anthers in the flowers, Anomocytic stomata in the leaves.
The (AEAP) of A.arvensis showed an anti-inflammatory effect in both 4mg/kg and 7 mg/kg doses compared to the negative control [$p<0.01$], while no statistically significant differences were noticed between the results of extract's groups and the results of the positive control group.

1. Introduction

Anagallis arvensis (Scarlet Pimpernel) is a common wild herb in the Middle East which belongs to the family Primulaceae [1] . It is grown in different environmental areas such as hills, lands and mountains [1][2]. *Anagallis arvensis* is a herbaceous plant that is 10 to 30 cm height. It has a ramified root, sessile and opposite oval-shaped to lanceolate-shaped leaves, small flowers with blue or orange petals [1][3]. Traditionally, the aerial parts are used externally for the treatment of infections [4]. The plant is reported to have an *in-vitro* anti-inflammatory, antibacterial, antiviral and anti-Candidiasis effects, in addition to enzyme inhibition activity towards tyrosenase, amylase and α-glucosidase [4][5][6][7][8]. Furthermore, the plant has an analgesic, anti-pyretic and hepatoprotective effects, *in-vivo* [9][10]. The plant contains a wide spectrum of active constituents such as Saponins, flavonoids, triterpenoids, tannins, and sterols [4][11]. Phenolic acids as Ferulic acid, Chlorogenic acid and Gallic acid were detected in the aerial parts [7]. In addition to several flavonoid and flavonoid glycosides such as quercetin, rutin, lanceoletin, balanitesin [12]. Cucurbitancines and cucurbitacine glycosides named Arvenin I, II, III, and Arvenin IV were also isolated from the whole plant parts [13]. Anagalline and desglucoanagalloside are triterpene saponins presented in the roots and the aerial parts [11]. The seeds are also found to contain alkaloids [11].

The plant is known to possess toxic properties [11][14]. The methanolic extract is cytotoxic to several cancer cell-lines [12], furthermore, Saponnins and triterpenoids are likely to have nephrotoxicity, hepatotoxicity and carcinogenicity effects [15]. Despite the toxicity of *A.arvensis*, this plant seems to occupy an Important place in research field as a medicinal plant, therefore, the present work aimed to detect some diagnostic characters for the plant powder, in addition to investigate the potential anti-inflammatory effect, *in-vivo* ,which in turn may be beneficial to determine whether the therapeutic activities occurs while applying safe doses or not.

2. Methodology

Plant collection:

The plant was collected from Karbalaa' countryside at the end of march. Some samples were at the flowering stage, while the others were carrying fruits. The plant was dried under shade in a well-ventilated room. After that the parts were grinded using an electrical grinder.

Microscopic identification:

The powder of each plant part was examined under an optical microscope using chloral hydrate as a medium for examination [16].

Preparation of extract:

The extract was prepared by pouring 8.12 g of dried grinded aerial parts in 80 ml ethyl alcohol 60% at 80 C° under a condenser for 2 hours. The extract was then filtered and evaporated on a water bath at 60 C° until dryness.

Acute anti-inflammatory effect:

Experimental animals:

Albino Wistar rats weighted between (120 to 200 gr) of both sexes were obtained from Karbalaa' university. The animals were housed under standard laboratory conditions at room temperature before experiment.

Acute inflammation induction:

Egg-white modal was used to induce an edema in the paw by subcutaneous injection [17]. The induction of inflammation was done one hour after the extract, saline solution and Diclofenac sodium were injected.

Study design:

Rats were divided into four groups each one containing six rats. Group I (negative control) received normal Saline. Group II (positive control) received Diclofenac sodium 10 mg/kg. Group III received 4 mg/kg extract, while Group IV received 7 mg/kg of extract. The rats were given the treatment intraperitoneally followed by induction of inflammation after 1 hour of treatment intake.

Evaluation of acute anti-inflammatory effect:

The paw volume was measured using a digital caliper directly before the inflammation induction and 1,3,5,24 hour, respectively after the induction.

The percentage of paw volume differences was calculated using this equation [18]:

$$\frac{V(x) - V(0)}{V(0)} X\ 100$$

V(0): Paw volume measured directly before inflammation induction (zero time)

V(x): Paw volume injected at the particular time.

Statistical study:

Data were presented as mean ± standard Deviation (StDev).The results were analyzed by One Way-ANNOVA using Statistical Package for the Social Sciences [SPSS] version 16.0. statistical significance was determined by Tukey test and LSD test. *P*-value less than 0.05 was considered as significant.

3. Results and discussion

Microscopic test results:

The microscopic test for roots, leaves, flowers and seeds showed several anatomical features. Some may be considered as diagnostic characters. They are listed in figure (1,2,3):

Roots diagnostic characters: showed the distribution of Starch granules which were simple, small spherical, with a small point hilum situated at the center (fig 1-A). Calcium oxalate crystals: they were cluster in shape found inside the parenchyma cells (fig 1-B). Xylem vessels were found singly or in small groups and they possessed reticulate or pitted walls (fig 1-D). The Collanchyme tissue was composed of yellowish- brown cells (fig 1-C).

Leaves diagnostic characters: The upper epidermis was composed of large cells with thin markedly sinuous walls in surface view. Stomata were anomocytic (fig 3-A). Fragments of epidermis and palisade cells was noticed in sectional view (fig 3- B).

Flowers diagnostic characters: Pollen grains which were small, spherical with three pores and smooth exine (fig 2- B). Fragments of the fibrous layer of the anthers showed characteristic thickening and beading of the walls (fig 2-A). Glandular trichomes came from the epidermis of the corolla or calyx were composed of one- or two-celled stalk and a spherical, unicellular head, or it may be composed of multi-celled stalk (fig 2-C and D).

Seeds diagnostic characters: The testa layer of the seeds was containing a reddish-brown pigments (fig 4- A). The endosperm cells with moderately thickened walls and show occasional small, intercellular spaces (fig 4-B). The fragments of the embryo, which were very abundant and composed of fairly small, thick-walled parenchyma (fig 4-C and D).

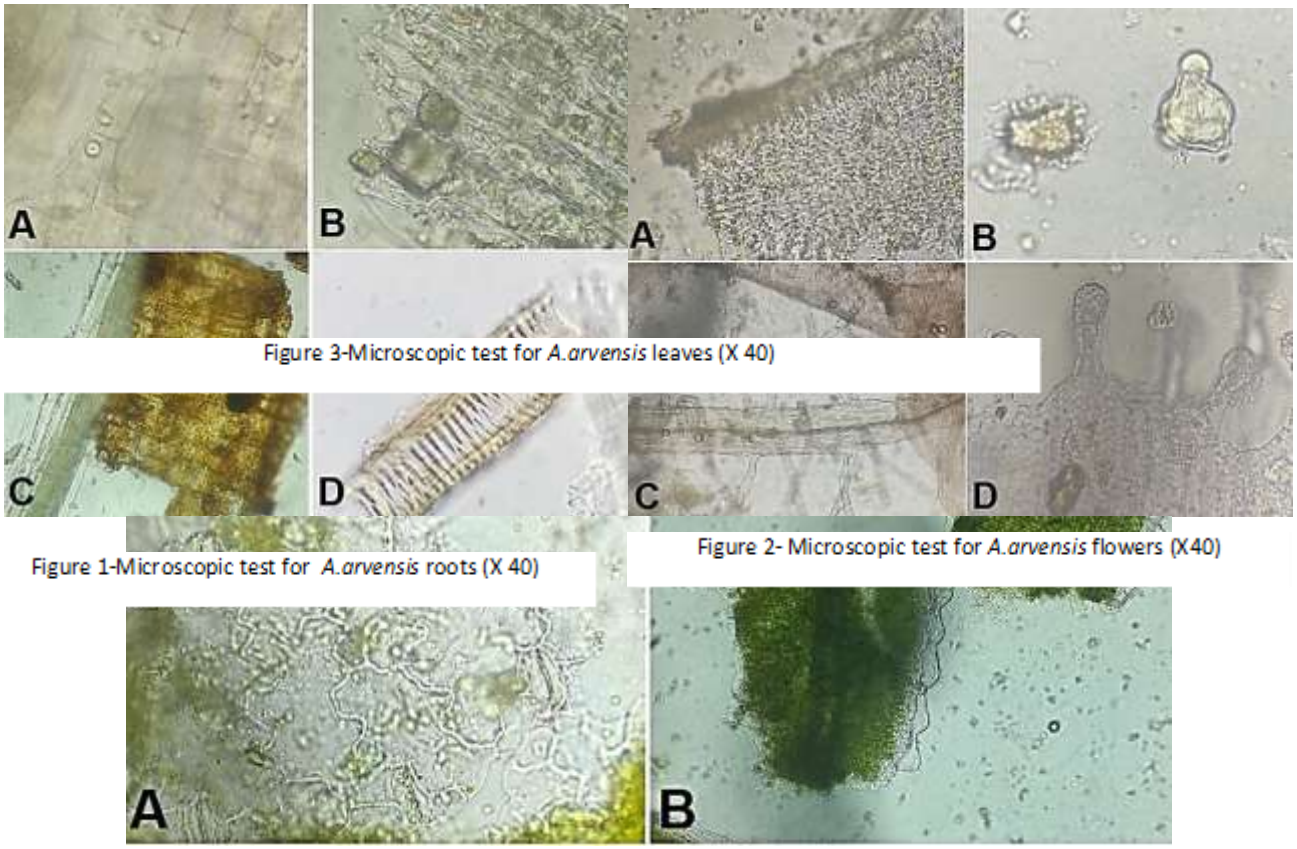

Figure 3-Microscopic test for *A.arvensis* leaves (X 40)

Figure 1-Microscopic test for *A.arvensis* roots (X 40)

Figure 2- Microscopic test for *A.arvensis* flowers (X40)

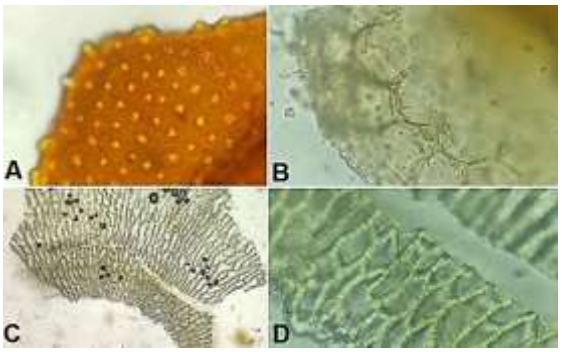

Figure 4-Microscopic test for *A.arvensis* seeds (X40 and X10 for C)

Anti-inflammatory activity evaluation results:

In egg-white modal, both the extract and Diclofenac sodium caused significant reduction in paw size ($P<0.05$ for the positive control and $P<0.01$ for groups of the extracts). The change in mean of paw diameter is shown in table-1 and the mean differences of paw diameter is shown in figure-5.

Table-1: Anti-inflammatory effect of the ethanolic extract from *Anagallis arvensis* using egg-white inflammation model in rats

Group [n=6]	**Mean of Paw diameter ± StDev**				
	Zero time	**1 hr**	**3 hr**	**5 hr**	**24 hr**
Negative control	5.05±0.45	7.19±1.2	7.04±1.18	5.98±0.92	5.35±0.18
Positive control	4.52±0.15	7.06±1.16	6.63±0.49	6.53±0.69	4.43±0.37*
4 mg/kg extract	4.63±0.37	7.23±1.10	6.97±1.01	6.26±0.74	4.19±0.46**
7 mg/kg extract	4.54±0.12	6.79±1.78	6.86±1.29	6.17±0.58	4.32±0.43**

(*: $P<0.05$ and **: $P<0.01$ compared to the negative control group. $\alpha=0.05$ and n= members in each group)

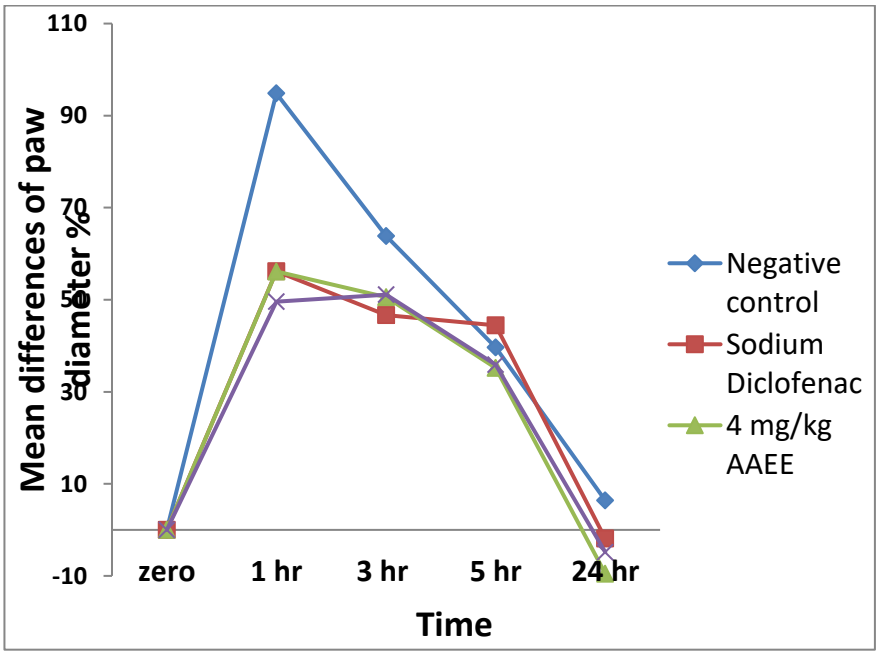

Figure-5: Effect of *A.arvensis* extract on the diameter of the paw in Wistar rats. Results are given as the percentage of mean differences of paw diameter.

A significant increase in paw diameter was noticed in all groups when measured 1 hour after induction ($P<0.01$). In comparison to the negative control, the differences between the mean of paw diameter measured in consecutive times for group 3, group 4 and the positive control showed no

significant anti-inflammatory effect after (1,3, and 5 hours) measurement (P>0.05) (table- 1), while, as it's also shown in table-1 and figure-5, a significant reduction of edema and paw diameter were observed after 24 hours measurement compared to the negative control (P<0.01 for 4 mg/kg group and 7 mg/kg extract group and P<0.05 for the positive control).

No significant differences were observed between the activity of the positive control and the extract, or between the two doses of the extract after 24 hours measurement (P>0.05) (table-1 and fig-5).

Discussion:

Several diagnostic characters of the aerial parts, roots and seeds were detected using the optical microscope, perhaps the most important of them are, the reticulate and pitted xylem vessels, Collanchyme tissue, anomocytic stomata, Pollen grains, the fibrous layer of the anthers, glandular trichomes, and the testa of the seeds.

Our results are in agreement with Abdel Moneim *et al*, 2003 in the term of pollen grains' shape and number of pores [3]. On the other hand, we detected several new diagnostic features of flowers and seeds, which we see so essential to identify the drug powder microscopically, especially, glandular trichomes and the fibrous layer as they were distinct in their shape.

The application of white-egg induced inflammation model is widely used to identify the acute anti-inflammatory activity of any drug [17].

The present study provides an evidence that the aerial parts of *Anagallis arvensis* have an anti-inflammatory effect, and the (Ethyl alcohol 60%) extract shows a significant reduction in edema and acute inflammation after 24 hours of I.P. injection. These results come in agreement with López *et al*, 2011 whose results revealed the anti-inflammatory effect of the methanolic extract for the aerial parts *in-vitro*, although we were the first to evaluate the anti-inflammatory effect for this plant *in-vivo* [4].

The lack statistical difference between the treatment groups and the positive control refers to a similar activity between *A. arvensis* used extract and Diclofenac sodium, however the activity should be compared with other NSAIds to obtain more comprehensive information about the effectiveness of the plant compared to medicines.

Our findings reveals that *A.arvensis* aerial parts contain saponins, alkaloids, phenolic acids, flavonoids and iridoid glycosides [7].

Anagallis arvensis methanol extract inhibits cyclooxygenase-1 and 2 *in-vitro* [4]. Cucurbitacines, such as cucurbitacin B exhibits an anti-inflammatory effect by down-regulating the levels of cytokines and inhibiting COX2 [19], in addition, triterpene saponins from several plants have an ant-inflammatory effect [20][21] .Furthermore, Flavonoids such as rutin have an anti-inflammatory effect through suppressing several mediators singling pathways [22][23]. Based on the above, we suggest that several active compounds could be involved in the anti-inflammatory mechanism of *A.arvensis* .

4. Conclusion and future scope

It can be concluded that *A. arvensis* parts contain several microscopic diagnostic characters, which some has been detected for the first time [e.g. the glandular trichomes and the fibrous layer]. Moreover, this study provides an important evidence that *A. arvensis* extract shows an anti-inflammatory effect. We suggest that further studies are needed to figure out whether the selected doses 4 mg/kg and 7 mg/kg may possess toxic effects or not.

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