Vol. 11/1 (2011) 76-78

JOURNAL OF NATURAL REMEDIES
Short Communication

The Haemagglutination potential of extracts from *Urtica dubia* plant parts.

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Abstract

The haemagglutination activity of protein extracts obtained from different plant parts of *Urtica dubia*, was tested *in vitro*. All three plant parts studied showed superior haeamagglutination activity after 60 minutes compared to a standard lectin, phytohaemagglutinin.

1.Objective of the study

The study was conducted to isolate an agglutinin extract from the rhizomes, leaves and stems of the stinging nettle (*Urtica dubia*), which is a member of the *Urticaceae* family, and to investigate the agglutination capacity of the lectin with time and concentration.

2. Plant material used

Urtica dubia (Urticaceae) plants were collected during November 2003 from the Southern part of Malta. After carefully washing with tap water, the plant parts were separated into rhizomes, leaves and stems.

3. Preparation of extracts / pure compound

The isolation of UDuA was based on a procedure described by Peumans and co-workers [1], with some modification. Briefly, the fresh plant materials (rhizomes, leaves and stems) were homogenised with 0.1N HCl (200 g/l) and allowed for 24 h shaking. The mixture was filtered and centrifuged at 5000 rev/min for 10 min. The pH of the collected supernatant was adjusted to 3.8 with 2N NaOH and allowed to react for 1 hour on an ice bath. The mixture was centrifuged again and the filtrate was collected for further analysis. $(NH_4)_2SO_4$ solution was added to the filtrate (3:2 respectively) and the mixture was centrifuged again at 5000 revs/ min for 20 min. The protein pellet was then dissolved in 250 ml of acetate buffer (50 mM Na acetate, pH 3.8 containing 0.1M NaCl).

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$(NH_4)_2SO_4$ solution was added again and the mixture centrifuged. The protein pellet was then prepared in phosphate buffer saline (PBS, Invitrogen) as serial dilutions ranging from 1 to 0.0001 %.

Phytohaemagglutinin (PHA, Invitrogen) was prepared likewise in PBS.

4. Tested activity

A suspension of red blood cells (RBCs) were prepared from fresh human blood, washed several times with PBS, by centrifugation at 2900 rpm for10 min during each washing. Trypsin solution (0.25 %) were added and the suspension washed again with PBS. The final RBC suspension concentration was 1 % [2]. 100 µl of RBC suspension were transferred to wells of 96 well plates, followed by 100 µl of untreated PBS, PHA and extracts, accordingly in triplicates. Four sets of plates were allowed to react for 20, 40, 60 and 80 minutes, individually. Finally the supernatants (150 µl) were removed from each well and residues were analysed on a STATFAX 2000 ELISA reader at a wavelength of 405 nm and a differential wavelength of 650 nm. 100 µl of 0.1 % solution of Triton® X 100 were added to each well and then mixed on a MTP shaker for 10 minutes at RT. The plates were subjected again to the ELISA reader at the same wavelengths. The % agglutination was calculated following the equation:

$$\text{Agglutination} = \frac{(\text{AverageAbsorbancebeforeTx})_{\text{treatment}} - (\text{AverageAbsorbanceafterTx})_{\text{treatment}}}{(\text{AverageAbsorbancebeforeTx})_{\text{control}} - (\text{AverageAbsorbanceafterTx})_{\text{control}}}$$

All data was analysed using ANOVA (one-way analysis of variance) and Bonferroni post-hoc test for the comparison of means with the control treatment [3] and ANCOVA (one-way analysis of co-variance) and two-tailed adjusted means T-test to determine time-related or concentration-related differences with control [4] with the BMDP/DYNAMIC v. 7.0 (Cork, Ireland) statistical package

5. Results

The results are reported in table 1.

Table 1: Agglutination values for *Urtica* leaves, roots and stems at a concentration range of 1 to 0.0001 % against PHA.

	PHA	Leaves	Roots	Stems
1	3.996 ± 0.259	4.594 ± 0.417*	4.693 ± 0.368*	4.824 ± 0.301**
0.1	3.926 ± 0.226	4.431 ± 0.267*	4.600 ± 0.327*	4.845 ± 0.550**
0.01	3.778 ± 0.319	4.207 ± 0.222*	4.469 ± 0.479*	4.773 ± 0.250**
0.001	3.751 ± 0.202	4.241 ± 0.530*	4.176 ± 0.354*	4.791 ± 0.416**
0.0001	3.765 ± 0.303	4.246 ± 0.562*	4.137 ± 0.480*	4.797 ± 0.465**

Each point is the mean ± SEM (ANOVA and Bonferroni post-hoc test: *p<0.1, **p<0.05, v=6 against PHA at each separate concentration).

6. Conclusion

Over the 80-min period, the best results were obtained after 60 min. This goes in accordance the time interval used by Longstaff and co-workers [2] in their experiments. Extracts from all three plant parts exhibited superior haemagglutination activity to the standard lectin, phytohaemagglutinin (Table 1). The highest activity was exhibited by the stems ($p<0.05$, $v=6$), followed by roots and leaves ($p<0.1$, $v=6$), as compared to PHA.

References

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