

AN EXAMINATION OF THE MUCILAGE-CONTAINING SEEDS OF *ALYSSUM CAMPESTRE* L.

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THE seeds known in Iran as "Ghoddumeh" are derived from *Alyssum campestre* L. (family Cruciferae)^{1,2}, a small annual herb, distributed throughout most parts of Iran. 3 botanical varieties have been described¹ and 2 kinds of these seeds are known in the Teheran market, one "Ghoddumeh shahri" (G. of town) and the other "Ghoddumeh shirazi" (G. of Shiraz). The latter are the most esteemed and are used especially as a mild expectorant.

The present investigation was undertaken to obtain information concerning the constituents of the seeds and to ascertain if they compared favourably, for pharmaceutical purposes, with other mucilage-containing seeds such as those derived from species of *Plantago*.

MATERIAL

Samples of seed used for the investigation were obtained in Teheran and consisted of ripe seeds with a small proportion of unripe seeds and about 2.5 per cent. of foreign seeds and other organic matter.

CHARACTERS OF THE SEEDS

Ripe seeds are pinkish-brown with a lighter margin, whilst the unripe seeds are olive-green with a margin of varying shades of yellowish-brown. Their size is very uniform varying from about 0.02 to 2.19 mm. in length and from 1.55 to 1.76 mm. in breadth. The seeds are very much flattened and broadly ovate to pear-shaped in outline. At the pointed end, the remains of a funicle are often present. A transparent V-shaped cleft occurs below the hilum to which runs an oblique groove, present on both sides of the seed. The testa is minutely pitted. The average weight of 100 seeds, selected at random, was 0.135 g., whereas that of 100 ripe seeds, selected by hand, was 0.150 g. On soaking a seed for a few moments in water the epidermis of the testa swells up and a thick mucilage soon surrounds it like a halo. When observed under a lens this mucilage, which is at first transparent, is seen to lose its transparency gradually while remaining translucent. Unripe seeds produce a smaller mucilage-halo than ripe seeds. Both the whole and powdered seeds are odourless and when chewed have a bland mucilaginous taste.

The powders produced from the ripe and unripe seeds are yellowish-brown and yellowish-green respectively. Small starch grains are abundant in the unripe seeds, but rare in those that have ripened. The mucilage stains with ruthenium red and with methylene blue, both stains being retained on washing with water. A pigment layer is present in the testa and preliminary experiments indicate its chief constituent to be an

THE SEEDS OF *ALYSSUM CAMPESTRE* L.

anthocyanin glycoside. The guaiacum resin and benzidine tests show a cold water extract of the powder to contain a peroxidase enzyme but no oxydase enzyme. On boiling the seeds with a solution of sodium hydroxide, a deep orange colour is produced.

EXPERIMENTAL

Swelling Factor. Mucilage-containing seeds are often evaluated by their swelling factors and previous work^{3,4} has shown that careful standardisation of the experimental conditions is essential if comparable results are to be obtained. In this case the following procedure was found to give satisfactory results. 1 g., accurately weighed, of seeds was placed in a 25 ml. graduated cylinder of diameter 1.5 cm. The water, or other aqueous solution, was added to the 20 ml. level and a plastic stopper inserted. The cylinder was vigorously shaken at the beginning of the experiment and again after 1, 2 and 20 hours. Immediately prior to each shaking the cylinder was inverted and gently tapped. The volumes of the swollen seeds were finally read at the end of 24 hours. Other determinations in which the cylinder was inverted once at the beginning of the experiment and in which subsequent shakings were omitted, were also carried out. The results are recorded in Table I.

TABLE I
SWELLING FACTORS UNDER DIFFERENT CONDITIONS AND USING DIFFERENT SOLUTIONS

Liquid	SWELLING FACTORS	
	With shaking	Without shaking
Distilled water	17.2—17.7—18.10	10.5
Chloroform water	17.0—17.5—18.0	9.5
Soft tap water	16.2—16.6—17.0	—
0.5 per cent. of sodium chloride	17.5	—
1.0 per cent. of sodium chloride	17.0	8.2
2.0 per cent. of sodium chloride	16.5	—
0.5 per cent. of potassium chloride	15.0	—
1.0 per cent. of potassium chloride	14.5	8.0
2.0 per cent. of potassium chloride	12.5	—
0.5 per cent. of calcium chloride	14.0	—
1.0 per cent. of calcium chloride	13.5	5.7
2.0 per cent. of calcium chloride	9.0	—

Isolation of the Mucilage. The complete extraction of the mucilage from the seeds presented problems which have not yet been completely solved. After many experiments with different presses and types of filter, the following general method was adopted.

The seeds, with 30 times their weight of distilled water were shaken frequently during 24 hours. The viscous product was poured, a little at a time into a linen cloth and the mucilage squeezed out by hand. The residual seeds were soaked for about 30 minutes in distilled water and the pressing repeated. This procedure was repeated 4 times. The mucilage from each pressing was precipitated separately by the addition of ethanol (95 per cent.) (6 ml. for every 5 ml. of mucilage) and stirred gently with a glass rod. In some instances, dilute hydrochloric acid or 1 per cent. sodium chloride solution was added to the mucilage prior to precipitation. The coagulated mucilage, which formed a mass floating on the dilute

ethanol, was transferred to a linen filter, allowed to drain and then pressed to remove all the dilute ethanol. It was dried at a temperature just below 100° C. and weighed. The product from the first extraction was white and in typical experiments represented 8.2 to 12.0 per cent. of the original weight of the seeds; the products of later extractions were dirty-white and formed 9.2 to 10.0 per cent. of the total seed weight. Two subsequent hydrations of the discoloured material in distilled water followed by precipitation with ethanol failed to yield an absolutely white product. When hydrochloric acid was used and the coagulated mucilage washed with ethanol and ether, white products with a fibrous appearance were obtained. The results are recorded in Table II.

TABLE II
EXTRACTION OF MUCILAGE FROM *Alyssum campestre*

Experiment	Weight of Seeds, g.	Volume of extracted mucilage, ml.	Volume of mucilage used for coagulation ml.	Dilute hydrochloric acid, ml.	1 per cent. sodium chloride solution, ml.	Ethanol (95 per cent.), ml.	Yield when dried in oven at 96° to 100° C., per cent.
I ..	5.0	420	420	—	—	505	17.4
II ..	7.5	580	580	1.5	—	695	19.8
III* ..	10.0	765	765	—	—	—	25.4
IV ..	5.0	450	{ 100 100 100 100	0.5	—	120 120 150 200	15.3 18.9 15.5 16.3
V ..	2.0	200	{ 100 100	— —	— 1	120 120	15.1 18.0
VI ..	3.0	240	{ 75 75 75	— — —	— 1 2	90 90 90	18.1 19.3 20.3

* In this experiment the total mucilage was evaporated and dried to constant weight at 100° C. The product was brown and brittle.

For the tests recorded below, the dried white mucilage was further purified by hydrating in the minimum quantity of distilled water on a water bath for 1 hour, cooling and adding the ethanol all at once with gentle stirring. To avoid any possible hydrolysis by heat or long standing⁵, the coagulated mucilage was immediately collected and dried *in vacuo*.

Properties of the Precipitated, Dried Mucilage. With water the precipitated and dried mucilage gives a neutral, slightly turbid, colloidal solution which is viscous in concentrations of 0.5 per cent. This solution does not reduce Fehling's solution until after hydrolysis with mineral acids. Addition of a solution of lead subacetate causes precipitation with the formation of a white gel on warming. There is no reaction with a borax solution.

The reconstituted mucilage requires more ethanol for its reprecipitation than originally. If the ethanol in the continuous phase is not sufficient, coagulation may occur at first, but later form a clear liquid on shaking. With a concentrated mucilage and a high proportion of ethanol, the coagulate is in a form which can be separated from the aqueous ethanol. The presence of small quantities of hydrochloric acid, sodium chloride,

THE SEEDS OF *ALYSSUM CAMPESTRE* L.

potassium chloride, calcium chloride and sodium sulphate make the mucilage more sensitive to coagulation by ethanol. The results of adding 1 ml. of a number of solutions to 5 ml. quantities of a 0.1 or 0.25 per cent. reconstituted mucilage are recorded in Table III.

TABLE III
ACTION OF REAGENTS ON MUCILAGE OF *Alyssum campestre*

Solution added, ml.	Strength of mucilage, per cent.	Result
Concentrated sulphuric acid	0.1	No coagulation
Sulphuric acid 10 per cent.	0.1	No coagulation
Sodium hydroxide 20 per cent.	0.1	Coagulation and no dispersal by shaking and standing
Sodium chloride 1 per cent. and 10 per cent.	0.1 or 0.25	No coagulation
Potassium chloride 1 per cent. and 10 per cent.	0.1 or 0.25	No coagulation
Calcium chloride 1 per cent. and 10 per cent.	0.1 or 0.25	No coagulation

Quantitative Determination of Mucilage by Greenberg's Method. The mucilage content of the seeds was determined by the method of Greenberg⁴ for seeds of *Plantago* spp. A weighed quantity of seeds were soaked in a definite volume of distilled water for 24 hours with vigorous agitation and transferred to the press. A seed:water ratio of 1:20 or 1:30 as employed with psyllium seeds, presented certain difficulties due to clogging of the sieve, incomplete removal of mucilage from the seeds and leakage of mucilage past the piston. These effects were largely overcome by reducing the seed:water ratio to 1:50 and by placing a small piece of linen over the sieve. A determination involving a reduced period of soaking with constant shaking was also made. The results, together with some of Greenberg's for comparison are shown in Table IV.

TABLE IV
MUCILAGE CONTENT OF THE SEEDS OF *Alyssum campestre* COMPARED

SEEDS	PERCENTAGE OF MUCILAGE			
	Seed : water ratio 1:20	Seed : water ratio 1:30	Seed : water ratio 1:50	Seed : water ratio 1:30. Soaking time 15 to 20 minutes
<i>Alyssum campestre</i> ..	13.00	16.00	19.50 ×	14 to 16
<i>Plantago ovata</i> ..	16.00	18.00	—	—
„ <i>arenaria</i> ..	* 7.66	* 7.36	—	—
„ <i>ovata</i> ..	*22.30	*21.70	—	—
„ <i>wrightiana</i> ..	*20.50	*23.00	—	—
„ <i>rhodosperma</i> ..	*16.00 (+)	*15.11 (+)	—	—

× In this case the soaked seeds were transferred to the press in two portions.
(+) Greenberg found that after further soaking and expression this species yielded about 18 to 20 per cent of mucilage.
* Figures obtained by Greenberg.

Miscellaneous Determinations on the Powdered Seeds

Moisture content—loss on drying at 100° C., 7.2 per cent.

Total ash—4.26 per cent., almost completely soluble in dilute hydrochloric acid.

Continuous extraction—with ether yielded 13.97 per cent. of extract.

Total nitrogen on the powder dried at 100° C., 3.06 per cent.

Sulphur determined by the U.S. Dispensatory (23rd edition) method 0.096 per cent.

DISCUSSION AND CONCLUSIONS

Table I indicates that chloroform water gives practically the same value for the swelling factor of *Alyssum campestre* as distilled water, but that low figures are obtained with salt-containing solutions and even with public water supplies. The swelling of the seeds is markedly reduced by the presence of sodium, potassium and particularly calcium ions and is inversely proportional to the concentration of the salt solutions. The need for the careful standardisation of the experimental conditions, if comparable results are to be obtained, is also confirmed.

The swelling factor of *Alyssum campestre* compares very favourably with the published figures for other seeds (Table V).

TABLE V
SWELLING FACTOR OF *Alyssum campestre* COMPARED WITH OTHER SEEDS,
(G) INDICATES GREENBERG'S FIGURES.⁴

SEEDS	SWELLING FACTOR	
	With shaking	Without shaking
<i>Alyssum campestre</i>	17.7	10.5
<i>Plantago arenaria</i>	17.0 (G)	8.0 (G)
„ <i>ovata</i>	11.0 (G)	9.5 (G)
„ <i>wrightiana</i>	20.0 (G)	14.5 (G)

To obtain the maximum yield of coagulated mucilage the minimum amount of water should be used for extraction and sufficient ethanol added to attain an alcoholic strength exceeding 50 per cent. (Table II). The yield of precipitated mucilage can also be increased by about 3.5 per cent. as a result of adding 2 ml. of 1 per cent. sodium chloride solution to each 100 ml. of mucilage. This increased sensitivity to ethanol is also obtainable by the addition of dilute hydrochloric acid.

Prolonged soaking of the seeds with successive quantities of water extracts other principles and renders the dried precipitated mucilage an off-white colour and difficult to purify. Naturally seeds containing earthy matter make purification of the mucilage still more difficult.

The quantitative determination of the mucilage content of the seeds by Greenberg's method⁴ is not entirely suitable since it is impossible to press out all the mucilage from the seeds after one soaking in water. Hence the mucilage values by this method were lower than the yield obtained by successive soakings with water and precipitation with ethanol. (Tables II

THE SEEDS OF *ALYSSUM CAMPESTRE* L.

and IV.) However, the mucilage content of the seeds, when determined by this method compares favourably with similar determinations on species of *Plantago*.

It would therefore appear that the seeds of *Alyssum campestre* contain sufficient mucilage to be of commercial value and in another publication⁶ the pharmaceutical applications have been investigated.

SUMMARY

1. The seeds of *Alyssum campestre* have been briefly described.
2. The extraction of the mucilage from the seeds and the properties of the mucilage have been investigated.
3. The proportion of mucilage in the seeds compares favourably with that in various species of *Plantago*.

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