THE ASSAY OF AMMI VISNAGA FRUITS

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In previous communications[1,2] a photoelectric colorimetric method is described for the assay of khellin in injections and tablets based on the intensity of the yellow colour which the chromone imparts to sulphuric acid of a definite concentration. In this work, the same method is applied for the assay of ammi visnaga fruits.

Abdel Rahman[3] has proposed a gravimetric method for the assay of ammi visnaga fruits based on exhausting the powdered drug by percolation with alcohol (95 per cent. v/v), removing the alcohol by distillation, diluting the concentrated extract with water, purifying it by treatment with lead acetate solution, removing the excess of lead from the filtrate with sodium dihydrogen phosphate, extracting the filtrate with chloroform, evaporating the chloroform, and weighing the dried residue. This residue was found by the authors to be impure and the results obtained by this method are higher than the actual quantity of non-glycosidal chromones present in the drug.

On replacing the alcohol (95 per cent. v/v), in the above method, by alcohol (70 per cent. v/v), or, on exhausting the powdered drug by boiling under a reflux condenser with alcohol (50 per cent. v/v), or water; no improvement in the purity of the residues could be obtained. On extracting these residues with boiling water, filtering, cooling the filtrate, extracting it with chloroform and evaporating the chloroform, a purer residue could be obtained. But on applying the photoelectric colorimetric method to such residues, it can be proved that they are still impure.

Experimental

Four quantities, each of about 5 g. of genuine ammi visnaga fruits, in moderately fine powder, are accurately weighed and separately exhausted by percolation with alcohol (95 per cent. v/v), alcohol (70 per cent. v/v), and by boiling under reflux with alcohol (50 per cent. v/v) and water, until the marc in each case fails to give a rose-red colour with a solution of sodium hydroxide (1 in 1).

From the alcoholic extracts, the alcohol is removed by distillation and each extract is diluted to about 100 ml. with distilled water; while the aqueous extract is concentrated to about 100 ml. Each extract is then separately treated with 10 ml. of a 10 per cent. solution of lead acetate, boiled and filtered while hot and the precipitate is washed on the filter with 3 quantities, each of 20 ml., of boiling water. To the combined filtrate and washings, 5 g. of sodium dihydrogen phosphate is added; after boiling, the precipitated lead phosphate is removed by filtration, and the precipitate on the filter washed with 3 quantities each of 10 ml. of boiling water. The filtrate and washings are concentrated in a porcelain

dish on the water-bath to about 50 ml. and filtered while hot into a separator. The dish and filter are washed with 3 quantities, each of 20 ml., of boiling water. The filtrate is cooled to room temperature, then extracted with 4 successive quantities, each of 25 ml., of chloroform. The combined chloroform extracts are washed with about 5 ml. of water, the water rejected and the chloroform extract is dehydrated with about 2 g. of anhydrous sodium sulphate and filtered through a dry filter paper into a tared flask. The sodium sulphate and the filter are washed with 3 quantities, each of 5 ml., of chloroform, adding the washings to the chloroform extract in the flask. The chloroform is then evaporated, the residue dried to constant weight at 100°C. and weighed.

The residues obtained are purified by extracting them separately with boiling water, filtering the aqueous solution while hot, cooling the filtrate to room temperature extracting with chloroform evaporation of the chloroform extract, drying the purified residues at 100°C. to constant weight and weighing. The amount of non-glycosidal chromones is determined in the residues thus obtained by the photoelectric colorimetric method[1,2], and calculated as khellin.

The mean results of three experiments for each method of extraction are given in Table I.

TABLE I

Method of Extraction	Percentage of residue	Percentage of purified residue	Percentage of chromones
Percolation with alcohol (95 per cent. v/v)	2·71	2·31	1·348
Percolation with alcohol (70 per cent. v/v)	2·57	2·16	1·538
Refluxing with alcohol (50 per cent. v/v)	2·71	2·23	1·558
Refluxing with water	2·64	2·04	1·629

From the above table it may be concluded that:—

(1) The residue obtained in the gravimetric method of assay of ammi visnaga fruits is impure.

(2) The different methods of extraction used did not help to obtain a pure residue.

(3) The method used for the purification of the residue did not succeed.

Therefore, the gravimetric method is unsuitable for the assay of ammi visnaga fruits as shown by the estimation of the chromones in the residues by the photoelectric colorimetric method.

Moreover, extraction of the fruits with boiling water gives better results than extraction with alcohol.

A Recommended Method for the Assay of Ammi Visnaga Fruits

From the foregoing investigation, it has been shown that the chloroform extract of an exhaustive decoction of ammi visnaga fruits is the most suitable for carrying out an accurate assay of the fruits, as the residue obtained by this method gives the highest colorimetric value and repre-

sents the true content of the non-glycosidal chromones (viz. khellin and visnagin) in the fruits.

Working with quantities of the drug not exceeding 0·5 g., the volume of the decoction resulting from the exhaustion of the fruits with boiling water is considerably reduced, making possible an easy application of the photoelectric colorimetric method for the assay of the fruits.

By this method the time of the assay is reduced to a maximum of 3 hours.

Procedure. Introduce about 0·25 g. of ammi visnaga fruits in moderately fine powder, accurately weighed, into a flask of about 150 ml. capacity; add 50 ml. of distilled water and boil the mixture under reflux for 30 minutes. Add to the boiling mixture 2 ml. of a 10 per cent. solution of lead acetate and continue the boiling for 3 minutes more. Filter the hot mixture by suction. Wash the flask and filter with 3 quantities, each of about 20 ml., of boiling water. Transfer the filtrate and washings to a beaker of about 250 ml. capacity; add 1 g. of sodium acid phosphate and boil for 3 minutes. Filter the hot solution directly into a separating funnel without suction. Wash the beaker and filter with 3 quantities, each of about 20 ml., of boiling water. Cool to room temperature. Extract the aqueous solution with 4 quantities, each of 25 ml., of chloroform. Wash the combined chloroform extracts with 5 ml. of water, reject the water, dehydrate the chloroform extract with about 2 g. of anhydrous sodium sulphate. Filter through a dry filter paper into a flask of about 200 ml. capacity; wash the sodium sulphate and the filter with 3 quantities, each of about 10 ml., of chloroform adding the washings to the chloroform extract in the flask.

Evaporate completely the chloroform on the water-bath. Add to the residue in the flask 80 ml. of 10 N sulphuric acid. Dissolve by the aid of gentle heat. Cool. Transfer the cooled acid solution to a volumetric flask of 100 ml. capacity. Make up to volume with distilled water. Mix well and leave to stand for about 5 minutes. Filter about 15 ml. of the solution into a dry colorimeter tube and read the percentage transmission of the solution at room temperature (25°C.) in a Lumetron Photoelectric colorimeter using blue filter 420 against water as the blank set at 100 per cent. transmission.

Find the concentration of khellin corresponding to the percentage transmission from a calibration table prepared at room temperature (25°C.) using standard concentrations of khellin. The figure obtained is the amount in mg. of the total non-glucosidal chromones contained in the weighed sample calculated as khellin. The percentage of these chromones in the drug can then be obtained by calculation.

Accuracy of the results. It has been found experimentally that the most accurate results are obtained on applying this method to weighed samples of the crude drug assaying about 4 mg. of khellin to get a reading of the per cent. transmission of the solution lying between 40 and 60. In this case, the results of the assay did not differ by more than $\pm$ 5 per cent.

Applying this method to different varieties of ammi visnaga fruits,

Table II shows the mean percentage of the non-glucosidal chromones calculated as khellin, resulting from 6 assays for each variety.

TABLE II

Variety	Percentage of chromones per cent.
Egyptian (Lower Egypt)	1·676
Egyptian (Upper Egypt)	1·530
Lebanon	1·120
Morocco	1·200

The above table shows that the fruits of the Egyptian variety of ammi visnaga are the richest in chromones.

SUMMARY

1. A recommended method for the assay of ammi visnaga fruits is described.

2. The results of the gravimetric method of assay of ammi visnaga fruits are high by not less than 50 per cent.

3. The fruit of the Egyptian variety of ammi visnaga contains the highest percentage of chromones.

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