A FURTHER NOTE ON THE GRAVIMETRIC DETERMINATION OF SANTONIN

By N. A. Qazilbash

From the Department of Botany, Islamia College, Peshawar

Received June 20, 1955

Artemisia is an important commercial drug. Several species of Artemisia contain santonin, which is a reliable specific for the elimination of intestinal roundworms. The important santonin-containing species of commercial value are, (i) *Artemisia Cina* Berg., (ii) *Artemisia kurramensis* Qazilbash, (iii) *Artemisia brevifolia* Wall, (iv) *Artemisia maritima* L., and (v) *Artemisia pauciflora* Weber.

In West Pakistan, *Artemisia kurramensis* Qazilbash and *Artemisia brevifolia* Wall, grow abundantly in commercial quantities in the Kurram and Gilgit Agencies respectively. Pakistan artemisias possess a great economic value in the world drug market on account of their high santonin-content.

The commercial value of artemisia is determined on the basis of its santonin-content. The assay of artemisia, therefore, is of considerable importance in assessing the market value of the drug. The writer has devoted much time and attention to the economic study of different species of Artemisia, growing in West Pakistan, Afghanistan and parts of Iran. A very large number of samples were collected, from time to time, during a period of about 30 years, from different geographical areas. The samples were thoroughly examined and assayed for their santonin-contents.

The amount of santonin in proportion to associated oily and resinous constituents is variable in different species of Artemisia. The relative proportion also varies in plants of the same species, growing in different ecological conditions.

The Assay of Santonin

As a result of extensive field tests at different altitudes in different geographical regions, and intensive laboratory work at Peshawar, in connection with the commercial utilisation of the Kurram and Kashmir artemisias, some slight modifications have been made in the author's assay method[1,2]. The important consideration in developing the technique, was to establish an economical, simple and reliable method of general application. The important modifications lately adopted are noted below: (i) the dried benzol extract is heated with the barium hydroxide solution on a steam bath, (ii) a stoppered Erlenmyer flask is used in place of a crystallising dish, (iii) the volume of the final filtrate from which the crystals of refined santonin, have been removed, is determined; and a correction-factor is added on the basis of 6·4 mg. per 10 ml. of the filtrate, to the weight of santonin finally obtained. The correction-factor takes into account the solubility of santonin in the ethanol solution

as well as the adsorption of santonin by the mixture of animal charcoal and kieselguhr.

Kassner *et al.*[3] have criticised the author's method[1] and have proposed an assay method, partly derived from the author's method and partly based on the assay method in use in their laboratory. They have adopted several modifications which may be summarised: (i) The use of bruised drug in place of the powdered material, (ii) percolation to exhaustion with the solvent, (iii) the use of saturated solution of barium hydroxide in place of 5 per cent. freshly prepared solution of barium hydroxide, (iv) the use of chloroform to dissolve the dried benzol extract before treating it with a saturated solution of barium hydroxide, and (v) the use of animal charcoal in place of a mixture of animal charcoal and kieselguhr.

The following comments are offered on the various points.

(i) *Use of the bruised drug in place of the powdered material.* Bruised material of *Artemisia brevifolia* Wall requires a greater volume of benzene than the powdered material to produce the required aliquot portion. When powdered material is used, the penetration of the alkali is more rapid and satisfactory and the subsequent extraction with benzene is adequate.

(ii) *Percolation to exhaustion.* Percolation to exhaustion requires a larger quantity of benzene than taking an aliquot portion after proper maceration which is more economical and gives satisfactory results. The writer has been concerned mainly with collections from remote hilly tracts, where transport is difficult and very expensive. An appreciable quantity of ballast matter is also carried away in the procedure, when the material is completely exhausted by large amount of the solvent. Maceration and taking an aliquot portion gives less trouble and the final yield is better.

(iii) *Use of a greater volume of saturated solution of barium hydroxide in place of 5 per cent. freshly prepared solution of barium hydroxide.* Kassner *et al.* consider 100 ml. of freshly prepared 5 per cent. solution of barium hydroxide insufficient for extracting all the available santonin from the benzol extract. They have suggested the use of a relatively greater volume of the saturated solution of barium hydroxide. Careful stirring with a glass rod brings about complete conversion of all the available santonin, when 110 ml. of freshly prepared 5 per cent. solution of barium hydroxide is used. The excess of barium hydroxide is undesirable. Theoretical calculations in regard to the chemical reactions involved do not justify the use of a greater volume of barium hydroxide solution, as suggested by them.

(iv) *Use of chloroform in dissolving the dried benzol extract.* When the dried benzol extract is dissolved in chloroform, and the resulting chloroformic extract is treated with a saturated solution of barium hydroxide as suggested by Kassner *et al.*, then the purity of the final yield of santonin suffers on this account. Better yield is obtained when the dried benzol extract is directly treated with the barium hydroxide solution.

(v) *Use of animal charcoal in place of a mixture of animal charcoal and kieselguhr.* Kassner *et al.* are of opinion that "kieselguhr is unnecessary

and only hinders filtration." Kieselguhr is universally known as filter-aid. Under normal weather conditions no hindrance in filtration is offered, when a mixture of equal parts of kieselguhr of suitable texture,

TABLE I

YIELDS OF SANTONIN AFTER USING KIESELGUHR AND CHARCOAL, OR CHARCOAL ALONE FOR CLARIFICATION

Artemisia species	Locality	Date of collection	Santonin per cent.	Melting point °C.	Composition of clarifying agent used
Artemisia kurramensis	Shublan	8.3.52	1·67	172.0	Mixture consisting of 2 parts kieselguhr and 3 parts animal charcoal
" "	"	"	1·68	172·1	Mixture consisting of 1 part kieselguhr and 1 part animal charcoal
Artemisia brevifolia	Rattu	15.8.50	1·37	173.0	Mixture consisting of 1 part kieselguhr and 1 part animal charcoal
" "	"	"	1·25	172·2	Mixture consisting of 2 parts kieselguhr and 3 parts animal charcoal
" "	Rampur	"	1·15	172·2	Mixture consisting of 1 part kieselguhr and 1 part animal charcoal
" "	"	"	1·14	172·0	Mixture consisting of 2 parts kieselguhr and 3 parts animal charcoal
Artemisia kurramensis	Parachinar	20.9.53	1·75	170·6	Animal charcoal alone
" "	"	"	1·85	172·0	Mixture consisting of 1 part kieselguhr and 1 part animal charcoal
" "	Zairan	22.9.53	1·41	171·2	Mixture consisting of 1 part kieselguhr and 1 part animal charcoal
" "	"	"	4·38	171·0	Animal charcoal alone
" "	Paiwar	"	1·27	172·2	Mixture consisting of 1 part kieselguhr and 1 part animal charcoal
" "	"	"	1·23	172·0	Animal charcoal alone
" "	Alizai	"	1·42	171·3	Mixture consisting of 1 part kieselguhr and 1 part animal charcoal
" "	"	"	1·38	171·0	Animal charcoal alone
" "	Islamia College	25.7.53	0·85	171·0	Mixture consisting of 1 part kieselguhr and 1 part animal charcoal
" "	"	"	0·84	170·0	Animal charcoal alone
" "	"	22.9.53	1·29	173·0	Mixture consisting of 1 part kieselguhr and 1 part animal charcoal
" "	"	"	0·98	173·2	Animal charcoal alone
" "	Nastikote	20.9.53	1·73	168·2	Mixture consisting of 1 part kieselguhr and 1 part animal charcoal
" "	"	"	1·69	167·0	Animal charcoal alone
" "	Islamia College	14.7.53	1·18	174·0	Mixture consisting of 1 part kieselguhr and 1 part animal charcoal
" "	"	"	1·15	173·2	Animal charcoal alone
" "	"	20.7.53	0·84	173·0	Mixture consisting of 1 part kieselguhr and 1 part animal charcoal
" "	"	"	0·84	172·0	Animal charcoal alone
" "	"	25.7.53	0·84	170·0	Animal charcoal alone
" "	"	"	0·85	170·4	Mixture consisting of 1 part kieselguhr and 1 part animal charcoal

and finely powdered animal charcoal is employed. In cold weather, when the temperature is low, a steam jacket could be used with advantage for rapid, efficient filtration. Kieselguhr is a good adsorbent, and its use is helpful in removing the colloidal resinous impurities, which otherwise escape removal.

Experimental evidence. Under controlled experimental conditions, samples of different species of Artemisia were assayed and the following reagents were employed as clarifying agents for the removal of impurities: (i) a mixture of 2 parts of kieselguhr and 3 parts of animal charcoal, (ii) a mixture of equal parts of kieselguhr and animal charcoal, (iii) animal charcoal alone.

The best results were obtained when a mixture of equal parts of kieselguhr and animal charcoal was used. When animal charcoal alone was employed, there was a greater loss of recoverable santonin, and there was noticeable deterioration in the final yield of santonin, as judged by colour and melting point. The results are given in Table I.

Experiments have also been made on the adsorption of santonin by (i) a mixture of equal parts of animal charcoal and kieselguhr and (ii) animal charcoal alone; under similar experimental conditions. The details are given below.

(i) *A mixture of equal parts of animal charcoal and kieselguhr.* A known quantity of pure santonin was boiled with 50 ml. of 15 per cent. ethanol w/w, under a reflux for 15 minutes, and filtered hot into an Erlenmeyer flask. The flask and the filter paper were washed three times with 5 ml. warm 15 per cent. ethanol. The filtrate was heated with 100 mg. of a mixture of equal parts of finely powdered animal charcoal and kieselguhr, under a reflux condenser for 10 minutes and filtered hot into a crystallising dish. The residue and the filter-paper were thrice rinsed with 5 ml. of the 15 per cent. ethanol. The crystallising dish was kept in the dark at 15°–17° C. for 24 hours. The crystals of santonin were collected on a weighed filter-paper (preheated at 103°–105° C. and cooled in a dessicator), and the crystallising and the filter-paper washed twice with 5 ml. of the 15 per cent. ethanol. The volume of the filtrate was determined. The crystals of santonin were dried at 103° to 105° C. and placed in a dessicator over sulphuric acid. The weight of santonin was then determined. The results are given below in Table II.

TABLE II

YIELD OF SANTONIN AFTER CLARIFICATION WITH EQUAL PARTS OF CHARCOAL AND KIESELGUHR

Weight of santonin taken (mg.)	Weight of santonin regained (mg.)	Loss in weight (mg.)	Volume of final filtrate (ml.)	Correction-factor per 10 ml. of the final filtrate
203·3	160·7	42·6	64·1	6·6
201·7	160·7	41·0	65·3	6·3
200·0	156·4	43·6	66·5	6·5
100·0	57·4	42·6	73·1	5·8
100·0	58·0	42·0	72·3	5·8

It was realised that the volume of the final ethanolic solution containing the refined santonin crystals, is noticeably influenced by weather conditions such as temperature, atmospheric humidity, and the movements of air-currents. In view of such considerations, a stoppered Erlenmeyer flask was substituted in place of the crystallising dish in later work. Two sets of experiments were carried out. In one case 100 mg. of a mixture

of equal parts of kieselguhr and animal charcoal was used and in the other case 100 mg. of animal charcoal alone was employed. The results are shown in Table III and Table IV respectively.

TABLE III

EXPERIMENT (A): A MIXTURE OF EQUAL PARTS OF KIESELGUHR AND ANIMAL CHARCOAL USED

Weight of santonin taken (mg.)	Weight of santonin regained (mg.)	Volume of the final filtrate (ml.)	Total loss in weight (mg.)	Loss in weight due to solubility in the filtrate	Other loss due to adsorption by the mixture of kieselguhr and animal charcoal
100	55·4	83	44·6	26·6	18·0
100	55·2	83	44·8	26·6	18·2
100	54·9	83	45·1	26·6	18·5
100	57·4	84	42·6	26·9	15·7
150	103·8	84	48·7	27·2	21·5
150	102·1	85	46·2	26·9	19·3
150	102·8	84	47·9	27·2	20·7
150	101·3	85	47·2	26·9	20·3
200	143·7	84	56·3	26·9	29·4
200	143·0	84	57·0	26·9	30·1
200	143·9	83	56·1	26·6	29·5
200	145·2	84	54·8	26·9	27·9

TABLE IV

EXPERIMENT (B): ANIMAL CHARCOAL ALONE USED

Weight of santonin taken (mg.)	Weight of santonin regained (mg.)	Volume of the final filtrate (ml.)	Total loss in weight (mg.)	Loss in weight due to solubility in the filtrate (mg.)	Other loss due to adsorption by animal charcoal (mg.)
100	34·8	84·0	65·2	32·8	32·4
100	38·0	83·0	62·0	32·4	29·6
150	87·8	83·0	62·2	32·4	29·8
150	85·5	83·5	64·5	32·6	31·9
200	129·5	83·5	70·5	32·6	37·9
200	131·3	83·0	68·7	32·4	36·3

Reference to Tables II, III and IV shows that there is a greater loss of santonin, when animal charcoal alone is used than when a mixture of kieselguhr and animal charcoal is employed. It is interesting to note that the adsorption of santonin is variable and inconsistent. The adsorption of colloidal resinous substances as well as santonin is influenced by the purity and degree of fineness of kieselguhr and animal charcoal. Due regard should therefore be paid to the standard quality and texture of the material used.

Badhwar *et al.*[4] have also shown that santonin adsorbed by animal charcoal is neither negligible nor consistent. They have recommended that treatment with animal charcoal be altogether avoided.

CONCLUSION

The assay method lately developed by Kassner *et al.*[3] is lengthy, time consuming, and requires large quantities of the solvent and reagents. The revised method proposed here is more economical and less time consuming; and the results are better qualitatively as well as quantitatively. The

final yield is white and has a melting point of 171° to 174° C. The method is also of general application. Also, with suitable adjustments, the method could be adopted for the isolation of santonin on an industrial scale. As a measure of normal routine the method gives the best results with good quality artemisias. Artemisias of low santonin-content deserve no serious consideration for purposes of commercial utilisation. Material totally devoid of santonin, or of poor quality, can easily be sorted out with the help of the potassium methoxide test. Experience with colour indications readily gives a clear idea of the approximate amount of santonin present. The chief interest of the author has been to arrange commercial collections of artemisia with high santonin-content from selected areas. The method could, however, be used for the quantitative determination of santonin in poor quality artemisias, by adopting slight modifications in the quantitative details. For this purpose, a larger quantity of the drug is taken, and suitable adjustments are made accordingly. At the final stage, the dried chloroformic extract is boiled with 50 ml. of ethanol (15 per cent. w/w), and proceeded with in the usual way. The amount of santonin could easily be estimated.

If enough artemisia is not in hand and only a limited quantity is available for assay purposes, the best alternative would be to mix the artemisia of poor quality with an equal quantity of best quality artemisia of known santonin-content (about 2 per cent.), and determine the amount of santonin in the mixture. The percentage of santonin in poor quality artemisia could be calculated.

SUMMARY

1. Some modifications have been made to the author's method of assay of artemisia reported in 1951.

2. Comments and experimental observations, relative to the various points raised by Kassner *et al.*,[3] are submitted.

REFERENCES

1. Qazilbash, *J. Pharm. Pharmacol.*, 1951, **3**, 105.
2. Qazilbash, *ibid.*, 1952, **4**, 511.
3. Kassner, Johnson and Terry, *ibid.*, 1953, **5**, 245.
4. Badhwar *et al.*, *Indian Forester*, 1951, **77**, 584.
5. Qazilbash, *Bull. Sci. Pharm.*, 1935, **33**, 131.