SCIENTIFIC SECTION

COMPARATIVE METHODS OF ASSAYS OF CHINESE EPHEDRAS.

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Seeing that the present methods of assays of Chinese Ephedras would not yield concordant figures for the percentage of total alkaloids of the same sample, the investigator has in view the finding of a suitable method, not too expensive nor too cumbersome, so that by preference such a method may be adopted as the standard.

In order to give a fair judgment of the quality of Chinese Ephedras, the Ephedras sample consisting of both *E. equisetina* and *E. sinica*—a mixture always obtainable from the Chinese market as the gatherer hardly knows nor cares for the difference in the drugs—were collected and dried in Tatung, Shansi, North China, by the Northwest Industrial and Trading Corporation of Tientsin, under the personal supervision of the investigator and were garbled to remove roots and foreign materials. The Ephedras were ground to powder, passed through a No. 20 sieve, and dried for seven days in a desiccator over sulphuric acid. The moisture content was found to be 4.7 per cent.

Before starting to experiment on methods, it might be mentioned that by applying the U. S. P. X method of assay for Belladonna alkaloids, only 0.87 of total base was obtained as the result of titration against 0.1 *N* HCl, using methyl red T. S.—the indicator used throughout the following experiments.

Experiment No. 1.—Mix, in a mortar or a dish, 5 Gm. of freshly slaked lime with 20 Gm. of Ephedras in powder, accurately weighed; add about 40 cc. of distilled water and incorporate until uniformly mixed and the powder is evenly moistened. Macerate the limed Ephedras for about ten minutes, and transfer the drug to a flask or, preferably, to a separatory funnel of about 500 cc. capacity. Dissolve 5 Gm. of ammonium chloride in 30 cc. of distilled water and add this solution to the drug in the funnel. After about five minutes, add 70 cc. of 95% alcohol and shake the mixture for fifteen minutes, then add 100 cc. of the alcohol in the same proportion, at a time; follow by vigorous shaking, as before, until 400 cc. has been added. Leave the mixture to macerate over night, or twelve hours, filter and return the drug to the same funnel. Instead of filtration, the alcoholic extraction can be cautiously run out from the funnel by slowly and carefully regulating the stop-cock. Evaporate the filtrate on a water-bath until there is no odor of alcohol and filter again into a second separatory funnel, carefully rinsing the filter with dilute ammonia water. The aqueous extract after being made strongly alkaline by the addition of 10 cc. of stronger ammonia water is shaken out three times for one hour each with chloroform 50, 40 and 40 cc., respectively. The chloroform extractions are evaporated *in vacuo* or, spontaneously, to dryness; the extract is dissolved in a little neutralized alcohol and titrated against 0.1 *N* HCl. There were used 16.32 cc. of 0.1 *N* HCl, indicating 1.3488% of total base.

The alcohol-extracted drug in the funnel is further frequently shaken out during three hours with 150 cc. of chloroform, 10 cc. of stronger ammonia and

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20 cc. of distilled water, being added prior to shaking. The chloroform extract is evaporated to dryness and titrated against 0.1 *N* HCl by first dissolving in a little neutralized alcohol, expelling same and filtering, if necessary. There were used 4.5 cc. of 0.1 *N* HCl, indicating 0.3719% of total base.

The twice-extracted drug is shaken out again during three hours with 100 cc. of chloroform and 10 cc. of stronger ammonia, and the chloroform extract is titrated against 0.1 *N* HCl as before. There were used 1.48 cc. of 0.1 *N* HCl, indicating 0.1223% of Ephedrine base. The total alkaloidal content was found to be 1.84%.

Experiment No. 2.—(*a*) Mix about 10 Gm. of freshly slaked lime with 20 Gm. of Ephedras in powder, accurately weighed, in a mortar or other suitable vessel; add about 50 cc. of distilled water, and incorporate until evenly moistened; macerate the mixture for about ten minutes and transfer the drug to a percolator. Pour on the drug 50 cc. of distilled water in which 3 Gm. of ammonium chloride has been previously dissolved, and, after about ten minutes, add 50 cc. of 95% alcohol. Macerate the mixture for about 12 hours, start the percolation at its natural rate and continue the percolation until 200 cc. of alcohol diluted (1–1) have been added and drained. Evaporate the percolate on a water-bath to drive off the alcohol and shake out the alkaloids, three times, during one hour, each with chloroform 50, 40 and 30 cc., respectively. The aqueous extract, after being filtered and rinsed with dilute ammonia, is made alkaline by adding 10 cc. of stronger ammonia water. Collect the chloroform extractions, filtering each portion if necessary; evaporate *in vacuo*, or spontaneously; dissolve the extract in a little neutralized alcohol, and titrate against 0.1 *N* HCl; 14.13 cc. of 0.1 *N* HCl were used, indicating 1.1678% of Ephedrine base.

(*b*) When the first percolation is finished, the drug in the percolator is immediately macerated with 30 cc. of distilled water, in which 3 Gm. of ammonium chloride has been dissolved, and, after ten minutes, followed by 70 cc. of 95% alcohol. After about 12 hours' maceration the percolation is carried through by the addition 200 cc. of diluted alcohol (alcohol 2, water 1). Proceed as before by evaporating the percolate, filtering, making alkaline, shaking out with chloroform, and titrating against 0.1 *N* HCl; 5.5 cc. of 0.1 *N* HCl were used, indicating 0.4545% of total base.

(*c*) After the second percolation is finished, the drug is further macerated with 20 cc. of distilled water, in which 4 Gm. of ammonium chloride has been dissolved; after 10 minutes, followed by 80 cc. of 95% alcohol and, after about 12 hours, the percolation is carried through by the addition of 200 cc. of diluted alcohol (alcohol 4, water 1). Proceed as before and titrate against 0.1 *N* HCl; 3.14 cc. of 0.1 *N* HCl were used, indicating 0.2595% of total base. The total alkaloidal content was found to be 1.88% in terms of Ephedrine base.

Experiment No. 3.—This is a repetition of No. 2 method, but instead of making three titrations, all three percolations are combined and titrated once for all. There were used 19 cc. of 0.1 *N* HCl, indicating 1.57% of total base.

Experiment No. 4.—This method is a modification of No. 1 but only allowing about three minutes each for the lime maceration and the lime ammonium chloride reaction before the addition of the alcohol. The alcoholic extraction yielded 1.0248% of total alkaloids.

After the alcoholic extraction, ether has been used for further extraction of the drug instead of chloroform and the result showed 0.4297% of Ephedrine base. The third maceration was accomplished by chloroform and 0.2562% of the base had been extracted. The total alkaloidal content by this method was 1.71% of Ephedrine base.

Experiment No. 5.—This is another modification of No. 1 method, allowing more time, about twenty minutes, for the lime reaction; thirty minutes for the lime ammonium chloride reaction, and forty-eight hours for the maceration; also doubling the quantity of lime and ammonium chloride, *i. e.*, 10 Gm. each. The alcoholic maceration yielded 1.457% of total base. After the alcoholic extraction, the drug in the funnel was further shaken out with chloroform as in case of No. 1 method and the chloroformic extraction was found to yield 0.377% of total base. The third extraction was performed with 100 cc. of chloroform and 10 cc. of stronger ammonia; the result was 0.15% of total base. The total alkaloidal content was found to be 1.984%.

The first and second titrations were reserved, shaken out with chloroform, and retitrated, requiring 13.69 cc. of 0.1 *N* HCl, showing 1.131% of total base.

Experiment No. 6.—This method is a modification of No. 2. The time allowed for reactions was prolonged to 15 minutes for the lime. Instead of adding the ammonium chloride separately, the ammonium chloride (2%) was dissolved in diluted alcohol (alcohol 95% 70 and water 30) and 100 cc. of this alcohol ammonium menstruum used to macerate the drug for about twelve hours, after the lime reaction, as in No. 2. The percolation was carried through and drained when 500 cc. of the same menstruum had been added and finished. The first percolation yielded 1.999% of total base by titration against 0.1 *N* HCl. After the first percolation, the drug was further extracted by adding 200 cc. of the same menstruum, after twelve hours' maceration. The second percolation extracted 0.056% of Ephedrine base. The total alkaloidal content was found to be 2.055%, highest of all the experiments.

The two titrations were reserved, shaken out with chloroform, and retitrated, using 14.77 cc. of 0.1 *N* HCl, indicating 1.22% of the total base. The large difference in the total base was believed to be due to the action of the atmospheric hydrochloric acid, which had partially neutralized the base as the chloroform extractions were not evaporated *in vacuo* but, spontaneously, in the air. This view was confirmed by the fact that during evaporation, nearing the end, the chloroform insoluble (almost) crystals of ephedrine hydrochloride were observed as white crystals floating on chloroform and also on the sides of the dish. Another fact might be mentioned that during the operation, distillation of hydrochloric acid had been going on, therefore, the acid was, naturally, carried by the atmosphere.

The fact has been proved by reshaking out with chloroform, after rendering the titrated solution strongly alkaline by caustic soda and stronger ammonia, evaporating the chloroform extractions *in vacuo*, and retitrating against 0.1 *N* HCl when 16.52 cc. had been used, indicating 1.365% of total base.

DISCUSSION.

While these results do confirm the high percentage of total alkaloids obtained by Feng and Read, and others; it, however, does not emphasize the need for hot

extraction, nor does it encourage the use of strong bases like soda and potash for liberating the alkaloids. A weak base like lime appears sufficient to liberate the alkaloids, but ammonium chloride and alcohol are essential to carry the alkaloids into solution. Both lime and ephedrine will liberate ammonia which will, in turn, further liberate the alkaloids and, partly, cause the formation of ephedrine hydrochloride, since ephedrine is a stronger base than ammonia and liberates it out from its salt. It is important to add the ammonium chloride, little at a time and constantly, as shown by the 6th experiment. Also the time allowed for the reactions is not a factor to be neglected.

TABLE OF RESULTS OF VARIOUS EXPERIMENTS.

Method.	Time allowed for reactions.	First extraction.	Reagents and solvents used.	Second extraction.	Reagents and solvents used.	Third extraction.	Reagents and solvents used.	Total base.
U. S. P. X Bellad. alkaloids	As specified	0.87%	As specified	.....		.....		0.87%
1st experiment	10 minutes for lime, 5 minutes for NH_4Cl and lime	1.3488%	Lime 5 Gm. NH_4Cl 5 Gm. alcohol 70 / water 30 — 500 cc.	0.3719%	Stronger NH_3 water, 10 cc., chloroform, 150 cc.	0.1223%	Stronger NH_3 water, 10 cc., chloroform, 100 cc.	1.84%
2nd experiment	10 minutes each for lime and NH_4Cl, lime	1.1678%	Lime 10 Gm. NH_4Cl 3 Gm. alcohol 1 / water 1 — 300 cc.	0.4545%	NH_4Cl 3 Gm. alcohol 20 / water 10 — 300 cc.	0.2595%	NH_4Cl 4 Gm. alcohol 80 / water 20 — 300 cc.	1.88%
3rd experiment	10 minutes each for lime and NH_4Cl, lime		Lime 10 Gm. NH_4Cl 3 Gm. alcohol 1 / water 1 — 300 cc.	.....	NH_4Cl 3 Gm. alcohol 20 / water 10 — 300 cc.	.....	NH_4Cl_4 Gm. alcohol 80 / water 20 — 300 cc.	1.57%
4th experiment	3 minutes each for lime and NH_4Cl and lime	1.0248%	Same as the first method	0.4297%	Stronger NH_3 water, 10 cc., ether, 150 cc.	0.2562%	Same as the first method	1.71%
5th experiment	20 minutes for lime, 30 minutes for NH_4Cl, lime	1.457%	Lime 10 Gm. NH_4Cl 10 Gm. alcohol 70 / water 30 — 500 cc.	0.377%	Stronger NH_3 water, 15 cc., chloroform, 150 cc.	0.15%	Stronger NH_3 water, 10 cc., chloroform, 100 cc.	1.984%
6th experiment	15 minutes for lime	1.999%	Lime 10 Gm. NH_4Cl, 2% alcohol 70 / water 30 — 600 cc.	0.056%	NH_4Cl 2% alcohol 70 / water 30 — 200 cc.	.....	.....	2.055%

CONCLUSION.

Experiment No. 6 may be recommended as the standard method; only 600-cc. percolation being necessary, as the second percolation does not yield appreciably high result.

The investigator wishes to announce that this report is open to critical discussions. Also this is only preliminary work on Mahuang, and further reports will follow in the course of time.

REFERENCES.

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