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A Study of the Assay of Ginger*

By Robert Tzucker† and C. B. Jordan‡

INTRODUCTION

Ginger has been known and used in medicine for a great many years. Although the use of ginger in medicine has fallen off to a great extent, it is still widely employed as a spice and in the preparation of various beverages.

The method of assay for ginger has never been a satisfactory one. The establishment of an accurate assay method is difficult due to the resinous consistency of the active constituents. The Ninth Revision of the United States Pharmacopœia was the first revision to include a standard for ginger. In this revision, the water-soluble extract, the non-volatile ether-soluble extract and the alcohol-soluble extract were determined. The Tenth Revision determined the water-soluble extract and the non-volatile ether-soluble extract. The Eleventh Revision determines the amount of ether-soluble extract, assuming, and rightly so, that the active or pungent principle is ether-soluble.

The method of the Eleventh Revision consists of extracting a portion of the drug with ether and weighing the residue after volatilization of the ether. Water-bath temperature is used to volatilize the ether. The objections to this method of assay are:

1. Individuals vary in their olfactory sensitivity to ether. Some operators may be unable to detect ether odor even though ether still remains in the extract; this, of course, will introduce an error in weight.

2. The active volatile principles may be partially but not entirely removed by heating at water-bath temperature, and therefore, the residue may not be a measure of all of the volatile constituents. Since the amount of volatile material coming off may vary, the weight of the residue will vary with different samples.

This study was carried out in an attempt to find a more satisfactory assay method.

HISTORICAL REVIEW OF THE METHODS OF ANALYSIS FOR GINGER

A method for the standardization of ginger was not introduced until the Ninth Revision of the United States Pharmacopœia. In that Revision the water-soluble extract, the non-volatile ether-soluble extract and the alcohol-soluble extract were determined.

Garnett and Grier (1909) used a shake-out method to determine the amount of gingerol, which has been shown to be the active principle. The method consisted of extracting the drug with ether, volatilizing the ether and boiling the residue with repeated portions of petroleum spirit. The petroleum ether is shaken out with successive portions of sixty per cent alcohol, leaving the volatile oil, fatty oil and much coloring matter in the petroleum spirit. The alcoholic solution is then washed with further portions of petroleum spirit to remove the last traces of fat. The alcohol is recovered or volatilized and the residual liquid shaken out with three successive portions of ether. The ether is then volatilized and the gingerol is dried to constant weight and weighed. Garnett and Grier (1907) claim that capsicum is a common adulterant of ginger. They give a method for determining the presence of capsicum. It consists of digesting on a water-bath about ten cc. of the extract with a small quantity of caustic alkali for fifteen minutes. The solvent is volatilized and the residue is acidified slightly with hydrochloric acid. It is then shaken out with a small portion of ether and the ethereal solution is tasted. In the case of pure ginger, the pungency will be found to have entirely disappeared, while if capsicum be present, the pungent, biting taste is at once recognized. One part of capsicum in one hundred parts of ginger may be detected in this manner.

Ginger extracts also give a specific color reaction. This test is carried out as follows:

Dilute 10 cc. of the extract to 30 cc.; evaporate to 20 cc. and extract this solution with 20 cc. of ether. Allow the ether to evaporate spontaneously in an evaporating dish and add 5 cc. of seventy-five per cent sulfuric acid and 5 mg. of vanillin to the residue. Allow this mixture to stand for about fifteen minutes and then add an equal volume of water. An azure-blue color develops in the presence of ginger extracts.

* An abstract of a thesis submitted to faculty of Purdue University in partial fulfilment of the requirements for the Master of Science degree, June 1938. The thesis contains a bibliography of fifty references.

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Clevenger (1928) claims that the method of the Ninth and Tenth Revisions of the United States Pharmacopœia of determining the volatile ether-soluble extractive is empirical and subject to several criticisms. These are:

1. The loss in weight does not always represent the volatile oil which usually is the desirable or active constituent; it may include volatile materials resulting from the decomposition of unknown substances.

2. The prolonged heating at 100° C. of the residue, which is necessary to remove the volatile matter completely from the ether-soluble extractive, increases the possibility of decomposition with subsequent erroneous results.

3. The volatile constituents of the product in question are not available for subsequent examination which is desirable in many instances.

Clevenger (1928) has devised an apparatus for the determination of volatile oils by means of which the amount of the volatile oil can be read directly. He has worked out standards for ginger and its preparations but the range of these standards seems rather wide. These are:

1. Volatile oil, 1.2 to 3.0 per cent.
2. Specific gravity of the volatile oil, 0.876 to 0.885.
3. Optical rotation of the volatile oil (25° C.) −40° to −56°.
4. Refractive index of the volatile oil (20° C.), 1.490 to 1.493.
5. Non-volatile ether-soluble extractive 3.5 to 7.1 per cent.
6. Iodine value of the non-volatile ether-soluble extractive 36 to 50.
7. Saponification value of the non-volatile ether-soluble extractive 45 to 70.

Clevenger claims that although the physical constants of the extractives vary considerably, the addition of appreciable amounts of an adulterant may be readily detected by deviations in the above mentioned constants.

EXPERIMENTAL PART

I. U. S. P. XI Method of Assay for Ginger.—In order to determine the accuracy of the U. S. P. XI method for the assay of ginger, several samples were analyzed by the official method. This consists of extracting a weighed portion of the drug, twenty Gm., with ether in a Soxhlet extraction apparatus for six hours. The liquid is then evaporated on a water-bath until the odor of ether is no longer perceptible. The container holding the extractive is then placed in a desiccator for twelve hours and weighed. The weight of the extract should be not less than nine-tenths of a Gm. or 4.5 per cent of the weight of the sample. This experiment was carried out on a sample of Jamaica ginger which had been stored in paper cartons in the basement for a period of eight months. Table I shows the results of this experiment.

Table I.—Ether Extractive

Twenty Gm. of Sample Used for Each Extraction

Sample Number	Per Cent of Residue	Weight of Residue
1	3.94	0.7870
2	3.94	0.7871
3	4.46	0.8932
4	4.56	0.9119
5	4.15	0.8299
6	4.22	0.8439
7	4.19	0.8389
8	4.21	0.8411
9	3.89	0.7786
10	3.93	0.7862
11	3.96	0.7913
12	3.95	0.7907
13	4.28	0.8560
14	4.28	0.8555
15	4.26	0.8510
16	4.26	0.8523

From this experiment, the fact is evident that this sample of ginger is not of Pharmacopœial strength and that concordant results are difficult to obtain by this assay method. A possible reason for the low results may be the loss of volatile material under the conditions of storage.

II. Effect of Water-Bath Temperature on the Ether-Soluble Extractive.—The next experiment was carried out to determine the effect of water-bath temperature on the residue over different periods of time. Since the sample of ginger used in the first experiment was not of U. S. P. strength, a fresh sample stored in an air-tight container was obtained. This sample of ginger was used in all of the remaining experiments. Table II shows the results of this experiment.

Table II.—Effect of Heating on Yield of Extractive

	Per Cent of Residue						
		After Heating on Water-Bath, Time in Hrs.					
Sample Number	After Spontaneous Evaporation of the Ether	1	2	3	4	5	6
1	4.92	4.55	4.32	4.22	4.15	4.10	4.02
2	4.88	4.53	4.18	4.09	4.03	3.96	3.88
3	4.87	4.50	4.12	4.01	3.90	3.84	3.79
4	4.86	4.43	4.19	4.11	4.00	3.93	3.88
5	5.02	4.54	4.26	4.16	4.02	3.94	3.86
6	4.98	4.62	4.34	4.21	4.07	4.01	3.92
7	4.99	4.58	4.21	4.13	3.96	3.87	3.80
8	4.87	4.41	4.22	4.10	3.98	3.84	3.76
9	5.21	4.62	4.50	4.37	4.31	4.26	4.19
10	5.10	4.56	4.39	4.22	4.12	4.05	3.98

By this experiment the fact is brought out that the length of time that the residue is exposed to water-bath temperature will greatly affect the weight of the residue. Two hours exposure changed all of the samples except one from U. S. P. strength to below the U. S. P. standard. This bears out the point previously mentioned, that the sensitivity of the individual in being able to detect the presence of ether will markedly affect the amount of residue obtained.

III. Volatility of the Ether-Soluble Extractive.—The next experiment was carried out to determine the volatility of the ether-soluble extract in air. Table III shows the results.

Table III.—Loss in Weight of Extract on Exposure to Air

Sample Number	Per Cent of Residue: After Spontaneous Evaporation of the Ether	After Exposure to Air, Time in Hrs. 5	12	36	72
1	4.73	4.70	4.68	4.54	4.52
2	4.74	4.72	4.69	4.66	4.64
3	4.79	4.77	4.75	4.72	4.70
4	4.77	4.74	4.72	4.70	4.67

The volatile constituents, as shown by this experiment, will volatilize to some extent at room temperature.

IV. Determination of the Non-Volatile Ether-Soluble Extractive.—Since the active constituent is reported as being non-volatile and ether-soluble, the next experiment was performed to determine the non-volatile ether-soluble extractive. The drug was extracted with ether as in the U. S. P. method and the ether permitted to evaporate spontaneously. An attempt was then made to dry the remaining residue to constant weight at 100° C., but, as Table IV shows, this was almost impossible because the residue lost weight indefinitely.

Table IV. Non-Volatile Ether Extractive

Twenty Gm. of Sample Used for Each Extraction

Sample Number	Per Cent of Residue: 1	2	3	4
After spontaneous evaporation of ether	5.02	4.98	5.00	4.88
In oven 60° C., 1 hour	4.81	4.77	4.84	4.65
In oven 100° C., 17 hours	3.66	3.60	3.69	3.57
In oven 100° C., 35 hours	3.50	3.44	3.51	3.34
In oven 100° C., 53 hours	3.42	3.34	3.41	3.25
In oven 100° C., 83 hours	3.30	3.25	3.32	3.10
In oven 100° C., 119 hours	3.23	3.18	3.24	3.02
In oven 100° C., 191 hours	3.05	3.02	3.02	2.85

The residue was tasted after 191 hours and there was no pungency present, showing either that the pungent principle is volatile at 100° C., or that it is oxidized to some non-pungent form.

V. Wirth's Method for the Determination of the Amount of Volatile Constituents.—E. H. Wirth of the University of Illinois College of Pharmacy, in a paper reported at the 1937 meeting of the American Association for the Advancement of Science, presented the following method for the determination of the total volatile material in ginger: He determined the moisture content by the toluene method. Then he determined the total volatile material by the oven method (dried the drug at 100° C. until there was no loss in weight). By subtracting the amount of moisture from the total volatile material, a value for the volatile material was obtained.

An attempt was made to follow this procedure. The amount of moisture was determined by the toluene method and was found to be 6.05 per cent. To determine the total volatile material the drug was weighed in small beakers and thus exposed to oven heat at 100° C. It was very difficult to weigh the drug as it absorbed moisture so rapidly that it was almost impossible to make an accurate weighing. The weighings were, therefore, made very rapidly but the results did not check. Glass stoppered weighing bottles were then substituted for the beakers. The drug was weighed and exposed for one hour periods in the oven. The results are shown in Table V.

Table V.—Volatile Extractive by Wirth's Method

Sample Number	Per Cent of Volatile Material: 1	2	3	4	5	6
Dried 1 hour at 100° C.	2.74	3.51	3.09	3.51	3.22	3.28
Dried 2 hours at 100° C.	5.32	5.56	5.09	5.27	4.87	4.96
Dried 3 hours at 100° C.	6.11	6.37	5.74	6.12	5.81	6.08
Dried 4 hours at 100° C.	4.24	6.48	5.94	6.28	6.04	6.22
Dried 5 hours at 100° C.	6.46	6.72	6.39	6.52	6.32	6.63
Dried 6 hours at 100° C.	6.71	7.06	6.64	6.73	6.61	6.94
Dried 7 hours at 100° C.	6.99	7.15	6.93	6.90	6.79	7.11
Dried 8 hours at 100° C.	7.07	7.19	6.98	7.01	6.92	7.17
Dried 9 hours at 100° C.	7.12	7.25	7.05	7.14	7.02	7.22
Dried 10 hours at 100° C.	7.20	7.33	7.11	7.27	7.12	7.30
Dried 11 hours at 100° C.	7.26	7.39	7.21	7.34	7.23	7.41
Dried 347 hours at 100° C.	9.26	9.39	9.33	9.19	9.34	9.46

Table V shows that it is almost impossible to dry the drug to constant weight; this seems to contradict Wirth's conclusions. Decomposition seems to take place and the drug becomes dark in color. It also loses its pungent taste. This drug was extracted in a Soxhlet extraction apparatus with ether for six hours but only a very small amount of extractive was obtained. The pungent principle apparently is volatilized or oxidized by heat. Since the drug could not be satisfactorily dried to constant weight, this line of experimentation was discontinued.

VI. Shake-Out Method for the Determination of Gingerol.—Garnett and Grier (1909) used a shake-out method to determine the amount of gingerol which has been said to be the chief constituent of ginger. They merely remove the fat and other materials from the ether extract by the use of various solvents. Gingerol has been shown to be somewhat soluble in these solvents and the result would not be an accurate measure of the quantity of gingerol. The procedure proved unsatisfactory in the hands of the operator because of the difficulty of separating the solvents used. Since this method was so closely related to that employed in the determination of the ether-soluble extract, with certain modifications, it was thought useless to pursue this line of investigation.

VII. Qualitative Test for Ginger Extracts.—Ten cubic centimeters of extract were diluted to 30 cc. This solution was evaporated to 20 cc. and then extracted with 20 cc. of ether. The ether was permitted to evaporate spontaneously in an evaporating dish and 5 cc. of a 75% solution of sulfuric acid and 5 mg. of vanillin were added to this residue. This mixture was permitted to stand for 15 minutes and then an equal volume of water was added. An azure-blue color appeared. This test is of value only in the testing for the complete absence of ginger extracts. If any amount of ginger extract is present, this blue color will be formed.

VIII. Clevenger's Method for the Determination of the Volatile Oil.—Clevenger (1928) devised an apparatus for the determination of the amount of

volatile oil in a drug. This apparatus was used and fairly good checks were obtained. Table VI shows the results.

Table VI.—Yield of Volatile Oil

Sample Number	1	2	3	4
Per cent of volatile oil	2.38	2.24	2.36	2.54

Apparatus for the determination of the other constants for ginger extracts given by Clevenger was of a special kind and could not be obtained. Since the procedure was quite long and involved, and could not be practically incorporated into the Pharmacopœia, this line of investigation was discontinued.

IX. New Method for the Determination of the Ether-Soluble Extractive.—In the U. S. P. XI the ether-soluble extractive is determined by weighing the residue from the ether extract after volatilization of the solvent. If a satisfactory method could be devised involving the weighing of the marc after ether extraction, more accurate results could probably be obtained. The next line of investigation was conducted with this end in view.

A paper extraction thimble was extracted with ether in a Soxhlet extraction apparatus for two hours; the ether was volatilized and the thimble weighed. The drug was then placed in the thimble and extracted with ether for six hours. The ether was volatilized from the extracted drug and thimble, and the thimble and extracted drug were then weighed. The loss in weight of the drug represented the ether-soluble extractive. Oven heat at 60° C. was used to volatilize the ether.

In the first attempts with this experiment, the thimble was weighed in air. This was quite difficult because the thimble absorbed moisture so rapidly that an accurate weighing could not be made. Next an attempt was made to use glass extraction thimbles but they proved to be unsatisfactory, so the use of paper extraction thimbles was again resorted to.

The next attempt involved the use of special glass-stoppered weighing bottles into which the extraction thimbles fit and thereby insure accurate weighings. The paper thimble was extracted with ether for two hours in a Soxhlet extraction apparatus; the ether was volatilized by the use of oven heat at 100° C., and the thimble was weighed in the closed weighing bottle. The drug, previously dried in a sulfuric acid desiccator for 48 hours, was then placed in the thimble and extracted with ether for six hours. The ether was volatilized and the thimble and drug weighed. The loss in weight of the drug represented the ether-soluble extractive. Table 7 shows the results obtained by this procedure.

Table VII.—Ether-Soluble Extractive by New Method

Sample Number	1	2	3	4	5	6
% ether-soluble extractive	4.58	4.69	4.77	4.70	4.67	4.53

SUMMARY AND CONCLUSIONS

1. The U. S. P. XI method for the assay of ginger is unsatisfactory. The reason for the failure of operators to obtain concordant results is mainly that the point at which all of the odor of ether is absent is almost impossible to ascertain. Different individuals also vary in their sensitivity toward the odor of ether.

2. Part of the ether-soluble extractive has been shown to be volatile.

3. Longer exposure of the ether-soluble extractive to water-bath temperature will cause a greater loss of weight. As a consequence, the amount of ether-soluble extractive in the sample of ginger may fall below the range of the standard of the U. S. P. XI.

4. Ginger should be stored in air-tight containers in a cool place.

5. The pungent material of ginger is either volatilized or oxidized at temperatures around 100° C.

6. Wirth's method for the determination of the volatile material proved unsatisfactory because the drug could not be dried to a constant weight.

7. Clevenger's method for the determination of the volatile oil content proved satisfactory. However, his methods for the determination of other constants of ginger extracts were too involved and necessitated the use of special equipment.

8. Ginger extracts give a specific color reaction.

9. A more satisfactory method of assay is proposed for the determination of the ether-soluble extractive. The procedure is as follows:

A paper thimble is extracted with ether for two hours in a Soxhlet extraction apparatus. The thimble is then placed in a special glass-stoppered weighing bottle, into which the thimble fits, and, with cover removed, placed in an oven at 100° C. for one hour to volatilize the ether. The cover is then put on the weighing bottle and it is cooled in a desiccator and weighed. The drug, previously dried in a sulfuric acid desiccator for 48 hours, is then placed in the thimble and weighed and is extracted with ether for six hours. The thimble and drug are then placed in the glass-

stoppered weighing bottle and, with cover removed, placed in an oven at 100° C. for one hour to volatilize the ether. The cover is then put on the weighing bottle and it is cooled in a desiccator and weighed. The loss in weight of the drug represents the ether-soluble extractive.

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NOTICE

Authors who desire to make corrections in papers read at the Richmond meeting, please do so promptly so that the manuscript may be sent to the Board of Review without delay.

Non-Interfering Adsorbents in Alkaloidal Analysis*

By Estelle Koozin Johnson and L. Wait Rising†

The purpose of this investigation was to find adsorbents that would be applicable in toxicological analysis involving alkaloids. An agent which coagulates protein material and removes pigments and dyes, but which does not adsorb any dissolved alkaloid, would not only facilitate filtration and clarification but would also eliminate digestion with heat and, therefore, would be especially applicable in analyses involving easily decomposable alkaloids.

Since the discovery by Lloyd (1), in 1910, of the affinity of fuller's earth for alkaloids, much work has been done toward finding an adsorbent which would adsorb alkaloids quantitatively. However, no systematic investigations have been made to determine which adsorbents have no affinity for all or some alkaloids and under what conditions the adsorption is at a minimum.

In this investigation, both the alkaloids and the adsorbents were selected so as to represent different chemical types, limiting the choice to the more commonly used alkaloids and adsorbents. The alkaloids were codeine, quinine, pilocarpine, atropine and cocaine. The adsorbents were talc, kaolin, prime silica gel, alumina cream and Activated Charcoal, U. S. P. XI. Charcoal was included to determine whether it has any selective action whatsoever, and to determine to what extent certain physical conditions affect its adsorptive capacity.

EXPERIMENTAL

General Procedure.—Aqueous solutions of the alkaloidal salts (0.5% and 1.0%) were prepared and the p_H adjusted to definite values. Five-gram and 20-gram portions of the adsorbents were placed in different bottles and 100-cc. portions of the alkaloidal solution were pipetted into each bottle.

* An abstract of a thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy, University of Washington, Seattle, Washington.

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To make the completion of this work possible, the Mellon Institute of Industrial Research, Pittsburgh, graciously provided laboratory space and equipment.