Effect of Ultrasonic Waves on the Extraction of Alkaloids

By M. E. OVADIA and D. M. SKAUEN

Cinchona bark, ipecac root, and jaborandi leaf were extracted with chloroform by means of a "step-horn" ultrasonic generator. Per cent total alkaloids extracted were plotted against time of extraction and results compared with conventional Soxhlet extraction. Results indicate that ultrasonic energy has an accelerating effect on the extraction of cinchona bark, ipecac root, and jaborandi leaf.

THE EFFECT of ultrasonic energy on the extraction of various components of plant cells has been investigated by a number of researchers (1–5). In a recent report, DeMaggio and Lott (6) described their research in the use of ultrasound for increasing alkaloidal yield. These authors made a clear distinction between ultrasonic maceration and ultrasonic extraction and concluded that ultrasonic energy definitely has utility in the extraction of alkaloid-containing plants, provided that the ultrasound is of sufficient intensity and is correctly applied.

In all of the reports reviewed on extraction of drugs using ultrasound, the sample to be insonated was immersed in an ultrasonic bath. The objective of this investigation was to determine the efficiency of a "step-horn" ultrasonic probe which can be inserted directly into the sample to be insonated.

EXPERIMENTAL

Material and Equipment.—*Cinchona succirubra*, No. 60 powder (0.250-mm. sieve opening), *Cephaelis ipecacuanha*, No. 80 powder (0.177-mm. sieve opening), and *Pilocarpus microphyllus*, No. 80 powder (S. B. Penick Co.); U.S.P. reagents; 8-fluid oz. polyethylene containers; Soxhlet extractors.

A 20-Kc. ultrasonic generator (model S-75, Branson Sonifier) was used in all extractions.

Procedure for Ultrasonic Extraction.—Accurately weighed samples of the crude drug were placed in 8 fluid-oz. polyethylene bottles and were allowed to macerate with the solvent mixture for the prescribed length of time. Two hundred milliliters of chloroform was added, and the ultrasonic step-horn was then immersed into the extraction mixture. It was held approximately 2.5 in. from the bottom of the container and 1 in. from the top. (Fig. 1.) Ultrasonic extraction was conducted for the desired period of time at the maximum power level. The extraction mixture was filtered, the residue washed with 40 ml. of chloroform in divided portions, and the combined filtrates mixed. The filtrates were concentrated and assayed by "The National Formulary" IX method for cinchona bark; the "United States Pharmacopeia" XVI method for ipecac root; and the "British Pharmaceutical Codex" 1949 method for jaborandi leaf. Results were compared with Soxhlet extractions.

Procedure for Soxhlet Extraction.—Accurately weighed samples of the crude drug were placed in Soxhlet thimbles and allowed to macerate with the solvent mixture for the prescribed length of time. Two hundred milliliters of chloroform was added and extractions effected for the desired period of time. The extracts were concentrated and assayed in the usual manner.

Procedure for Ultrasonic Extraction under Controlled Temperature Conditions.—The extraction mixtures were prepared as described under *Procedure for Ultrasonic Extractions*. However, the mixtures were cooled to 20° before insonation and immersed in an ice bath at 2° during insonation. Resulting extracts were processed and assayed as above and comparisons made between the three extraction techniques.

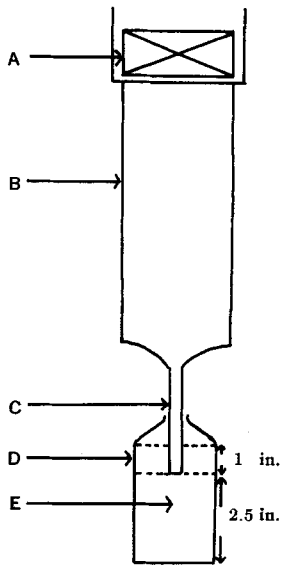

Fig. 1.—Position of the step horn of the S-75 Sonifier with relation to sample during ultrasonic extraction. Key: A, transducer; B, sonic converter, C, step horn; D, polyethylene bottle; E, extraction mixture.

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TABLE I.—PER CENT TOTAL ALKALOIDS OF CINCHONA EXTRACTED WITH CHLOROFORM USING VARIOUS METHODS OF EXTRACTION

Extraction Time, hr.	Soxhlet Extraction	Ultrasonic Extraction	Temp., °C.	Ultrasonic Extraction Under Controlled Temp.	Temp., °C.
0.5	...	6.25	55	5.57	25
1	5.38	6.83	55	6.37	25
1.5	...	7.15	55	6.61	25
3	6.16	7.28	55	6.80	25
5	6.42	...	..	...	..
7	6.65	...	..	...	..
Crude drug assay	6.85				

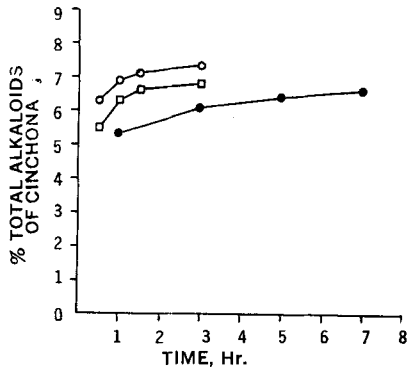

Fig. 2.—Extraction of cinchona bark with chloroform using various procedures. Key: O, ultrasonic extraction; □, ultrasonic extraction under controlled conditions; ●, Soxhlet extraction.

RESULTS AND DISCUSSION

Extraction of Cinchona Bark.—Accurately weighed 15-Gm. samples of cinchona bark powder were allowed to macerate with 35 ml. of a solution composed of 20 ml. of ether, 10 ml. of alcohol, and 5 ml. of ammonium hydroxide for 15 min. Extraction was conducted in duplicate by the methods described above, using 200 ml. of chloroform. Ultrasonic extractions were conducted for 0.5, 1, 1.5, and 3 hr. Soxhlet extractions were conducted for 1, 3, 5, and 7 hr. Results are shown in Table I and Fig. 2.

It is evident from the data that greater alkaloidal yield resulted from ultrasonic extraction without temperature control than by either Soxhlet extraction or temperature-controlled ultrasonic irradiation. One-half hour of ultrasonic extraction is nearly equivalent to 3 hr. of Soxhlet extraction. Forty-five minutes of ultrasonic treatment was nearly equivalent to 7 hr. of Soxhlet extraction. This compares favorably with results obtained by Head *et al.* (3) using a bath-type ultrasonic arrangement.

Extraction of Ipecac Root.—Accurately weighed 5-Gm. samples of ipecac root powder were allowed to macerate with 20 ml. of ether for 10 min. Three milliliters of ammonium hydroxide was added, mixed thoroughly, and the mixtures allowed to stand for 15 min. Extraction was conducted in duplicate by the methods described above using 200 ml. of chloroform. Ultrasonic extractions were conducted for 0.25, 0.5, 1, 3, 5, and 15 min. and 0.5, 1, 1.5, and 3 hr. Soxhlet extractions were conducted for 0.5, 1, 3, 5, and 7 hr. Results are shown in Table II and Fig. 3.

The results were most dramatic in this instance. Within 0.5 min., the alkaloidal concentration produced by ultrasound was greater than 5 hr. of Soxhlet extraction. In about 5 min., complete extraction seemed to have taken place.

Extraction of Jaborandi Leaf.—Accurately weighed 10-Gm. samples of jaborandi leaf powder were allowed to macerate with 50 ml. of chloroform for 10 min. Five milliliters of ammonia T.S. was added, mixed thoroughly, and allowed to stand 15 min. Extraction was conducted in duplicate by the methods described above using 150 ml. of chloroform. Ultrasonic irradiation was performed for 0.25, 0.5, 1, 3, 5, 10, and 15 min., while Soxhlet extraction went for 0.5, 1, 3, 5, and 7 hr. Results are shown in Table III and Fig. 4.

These indicate that 15 sec. (0.25 min.) of ultrasonic treatment extracted more alkaloids of jaborandi leaf than 5 hr. of Soxhlet extraction. Cooling the insonated liquid mixture appeared to be beneficial since a higher yield was produced in 0.5-min. period, and the extracts seemed to degrade at a slower rate. By all three extraction methods, there was evidence of degradation under the conditions of the experiments. This may be due to an opening in the lactone ring of the principal alkaloids during the extraction process in an alkaline medium (7).

CONCLUSIONS

1. A step-horn-type ultrasonic probe is capable of aiding in the extraction of alkaloids from certain crude drugs.

2. In all cases studied, the rate of extraction was more rapid with ultrasound than with Soxhlet extraction.

3. The maximum amount of alkaloids obtained by ultrasonic extraction of cinchona bark and ipecac root, and ultrasonic extraction of jaborandi leaf under temperature control was higher than the amount of alkaloids extracted in the official method of assay according to the N.F. IX, U.S.P. XVI, and B.P.C. 1949, respectively.

4. Ultrasonic extraction of cinchona bark under controlled temperature conditions lowered its rate of extraction compared to ultrasonic extraction at insonation temperatures. However, ultrasonic extraction of ipecac root under temperature control had no effect on its rate of extraction.

5. The amount of alkaloids extracted from ipecac root in 30 sec. was greater than the amount produced by Soxhlet extraction in 5 hr.

6. Alkaloidal extraction from ipecac root reached a maximum in about 5 min. using ultrasound and in about 5 hr. with Soxhlet extraction. Ultrasonic extraction yielded higher amounts of alkaloids,

TABLE II.—PER CENT TOTAL ALKALOIDS OF IPECAC EXTRACTED WITH CHLOROFORM USING VARIOUS METHODS OF EXTRACTION

Extraction Time	Soxhlet Extraction	Ultrasonic Extraction	Temp., °C.	Ultrasonic Extraction Under Controlled Temp.	Temp., °C.
0.25 min.	...	0.79	27	0.785	20
0.5 min.	...	0.895	29	0.90	20
1 min.	...	0.91	31	0.92	20
3 min.	...	0.945	41	0.955	23
5 min.	...	0.96	46	0.96	25
15 min.	...	0.96	52	0.95	25
0.5 hr.	0.214	0.95	55	0.965	25
1 hr.	0.38	0.96	55	0.95	25
1.5 hr.	...	0.96	55	0.96	25
3 hr.	0.71	0.95	55	0.96	25
5 hr.	0.84	...	..	...	..
7 hr.	0.83	...	..	...	..
Crude drug assay	0.83				

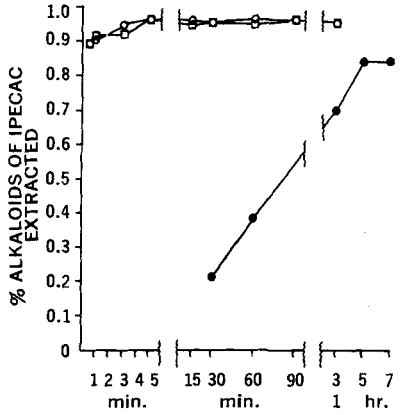

Fig. 3.—Extraction of ipecac root with chloroform using various procedures. Key: O, ultrasonic extraction; □, ultrasonic extraction, temperature controlled; ●, Soxhlet extraction.

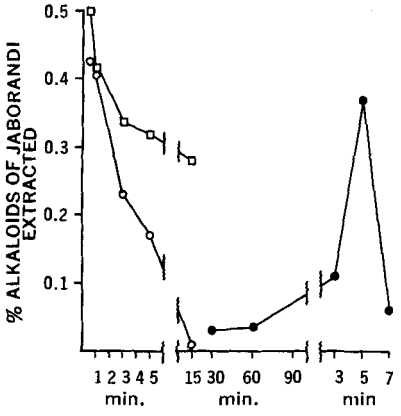

Fig. 4.—Extraction of jaborandi leaf with chloroform using various procedures. Key: O, ultrasonic extraction; □, ultrasonic extraction under controlled conditions; ●, Soxhlet extraction.

TABLE III.—PER CENT TOTAL ALKALOIDS OF JABORANDI LEAF EXTRACTED WITH CHLOROFORM USING VARIOUS METHODS OF EXTRACTION

Extraction Time	Soxhlet Extraction	Ultrasonic Extraction	Temp., °C.	Ultrasonic Extraction Under Controlled Temp.	Temp., °C.
0.25 min.	...	0.41	27	0.49	20
0.5 min.	...	0.425	28.5	0.50	20
1 min.	...	0.404	31	0.32	20
3 min.	...	0.23	41	0.355	23
5 min.	...	0.17	46	0.32	25
10 min.	...	0.09	52	0.30	25
15 min.	...	0.01	35	0.28	25
0.5 hr.	0.03	...	...	...	...
1 hr.	0.035	...	...	...	...
3 hr.	0.11	...	...	...	...
5 hr.	0.37	...	...	...	...
7 hr.	0.06	...	...	...	...
Crude drug assay	0.46				

7. Fifteen seconds of ultrasonic treatment resulted in greater alkaloidal yields from jaborandi leaf than 5 hr. of Soxhlet extraction.

8. The amount of alkaloids extracted from jaborandi leaf reached a maximum after 0.5 min. of ultrasonic extraction and after 5 hr. of Soxhlet extraction. Ultrasonic extraction yielded higher amounts of alkaloids.

9. Jaborandi leaf alkaloids showed degradation after 0.5 min. of ultrasonic extraction and after 5 hr. of Soxhlet extraction.

10. The rate of degradation of jaborandi leaf alkaloids under the influence of ultrasonic irradiation was faster than under controlled temperature conditions.

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Atromentin

Anticoagulant from *Hydnellum diabolus*

By JATINDER M. KHANNA*, MARVIN H. MALONE, KENNETH L. EULER, and LYNN R. BRADY

Gross screening revealed that *Hydnellum diabolus* Banker contained a parenterally effective anticoagulant, while *H. aurantiacum, H. caeruleum,* and *Hydnum laevigatum* lacked activity. A 70 per cent ethanolic extract of *H. diabolus* contained all anticoagulant activity as well as pressor-leiomyotonic principles. The anticoagulant activity grossly resembled that of heparin, except that *in vivo* activity was not reversed by protamine injection. *In vivo,* 1 mg. of the ethanol extract was equivalent to 0.58 u. of heparin. Selective solvent extraction of the mushroom with diethyl ether removed all anticoagulant activity, and from this extract atromentin was isolated. *In vitro,* 1 mg. of atromentin was equivalent to 5.1 u. of heparin and 2.3 mg. of reference 70 per cent ethanol extract. Synthetic dimethylatromentin, 2,5-diphenylbenzoquinone, and polyporic acid were without anticoagulant properties.

HYDNELLUM DIABOLUS Banker is widely distributed from Maine to Alabama and in the Pacific Northwest of the United States (1). While possessing an intense acrid taste and the odor of hickory nuts, it is generally regarded as edible. Until recently (2), pharmacologic activity had not been reported for this species.

EXPERIMENTAL

Collection and Preparation of Plant Material.—Carpophores of *H. diabolus* Banker were collected on Whidbey Island in western Washington on November 1, 1962. After cleaning, they were dried in a forced-air drying oven at 45° for at least 72 hr. A sample of the dried mushroom was pulverized in a ball mill overnight and passed through a 200-mesh sieve. A 70% ethanolic extract was prepared from another sample of this collection (60-mesh powder) using 30 parts of solvent per unit weight of mushroom and shaking on a reciprocal shaker for 20–24 hr. with 3 successive portions of solvent. The ethanolic extracts were separated by suction filtration, combined, and evaporated to dryness under reduced pressure at a temperature not exceeding 45° (37% extractive). This product was termed reference 70% ethanol extract. The marc was freed of solvent and reduced to a 200-mesh powder in a ball mill. The powdered whole carpophores, extract, and marc were coded and submitted for routine pharmacologic examination. Immediately prior to testing, all test materials were dissolved and/or suspended by trituration with 0.25% agar.

Detection of pharmacologic activity in *H. diabolus* suggested the desirability of testing other available members of the *Hydnaceae*. In a similar way, samples representing the powdered whole carpophores, ethanol extracts, and marcs were prepared for *H. aurantiacum* (Fr.) Karst., *H. caeruleum* (Pers.) Karst., and *Hydnum laevigatum* Fr. These mushrooms were collected in western Washington during the autumns of 1962 and 1963. The yields of 70% ethanol extractives were 36, 32, and 50%, respectively. Taxonomic identification of all the species was provided by Dr. D. E. Stuntz, Professor of Botany, University of Washington.

Test Animals.—Albino rats were obtained from E. G. Steinhilber, Oshkosh, Wis., and maintained on an unrestricted diet of laboratory chow (Purina) and water for at least 2 weeks before testing. Unless specified, all intraperitoneal injections were made into the upper left quadrant of the abdomen. Dogs used as blood donors and for pharmacodynamic testing were healthy mongrels purchased from local sources.

Qualitative and Semiquantitative Hippocratic Screening.—Using log-dosages from inactive to lethal, the test materials were injected intraperitoneally into male and female (150–250 Gm.) rats that had not received any previous drug treatment. Animals were not fasted prior to medication, and

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* Present address: Department of Pharmacology, Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada. Grantee: 1962–64 Lederle Fellowship for Natural Product Pharmacology.