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The effect of ultrasonic energy on the extraction of anthraquinones from senna pericarps

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Ultrasonic irradiation increased the extraction rate of rhein glycosides, free anthraquinones and total water-soluble solids from whole senna pericarps, irrespective of the temperature conditions employed. An increase in ultrasonic power from 35 to 100 W increased the yield of extractable constituents over a 15 min period, the ratio of rhein glycosides to total water-soluble solids being higher in extracts prepared using ultrasound than in preparations obtained by simple maceration, but that ratio decreased slightly with an increase in ultrasonic power. Ultrasonic irradiation produced occasional visible damage to epidermal cell walls and the significance of this in the extraction of senna pericarps is discussed.

Among those factors considered to be involved in the ultrasonic extraction of plant material are dispersion of adhering material, particle size reduction, partial cell wall disruption, gross stirring effects, selective agitation at phase boundaries, thermal effects and liberation of active constituents from bound sites within the cell (Boswart & Blazek, 1954; Thompson & Sutherland, 1955; Golian & Tamas, 1956; Drabent & Podeszewski, 1958; Wray & Small, 1958; DeMaggio, Lott & Gerraughty, 1963).

Morrison & Woodford (1967) showed that ultrasonic irradiation of an aqueous suspension of senna pericarps produced an increase in the extraction rate of both sennosides and free anthraquinones over a 90 min period. The temperature rise produced by insonation accounted for about 70% of the increase in those constituents and the hydrogen peroxide formed in the extraction liquid was not, apparently, a factor in the specific effect of ultrasonic energy.

In the present work the effect of ultrasonic irradiation on the extraction of senna pericarps has been examined more fully to seek possible explanations for the results obtained, and to determine the effect of ultrasonic power on that process.

EXPERIMENTAL

Effect of temperature

Ultrasonic irradiation of aqueous suspensions of whole senna pericarps was carried out as described by Morrison & Woodford (1967), 12 ml samples of the extract being withdrawn after 15, 30, 45, 60, 75 and 90 min into empty test tubes immersed in ice-water and the content of free and combined anthraquinone derivatives determined in 10 ml at 20°. The remaining 2 ml was returned to the extraction vessel and the 10 ml volume removed at each time interval was replaced by 10 ml of purified water, the solvent volume being maintained constant throughout. The temperature increase was noted and the experiment repeated in the absence of ultrasonic energy using a stirrer-hot plate apparatus to duplicate the temperature rise. Stirring was

necessary to produce an even temperature rise but preliminary experiments showed that the rate of stirring had no effect on the yield of extractable constituents.

To determine the effect of insonation at room temperature, the normal ultrasonic technique was employed except that the extraction vessel was partly immersed in an ice-salt mixture maintained at −5°. By controlling the degree of immersion of the extraction vessel in this mixture the temperature of the liquid in the beaker was maintained at 20 ± 0·5°. The experiment was repeated at room temperature (20°) by replacing the ultrasonic probe with a glass stirrer and omitting the cooling system.

The rhein glycoside content of the aqueous senna extracts was determined as described in Appendix II of the Recommended Methods for the Evaluation of Drugs: The Chemical Assay of Senna Fruit and Senna Leaf (1965) after removal of the free compounds using anaesthetic ether. The amount of free anthraquinones in the ether was determined after extraction with N sodium hydroxide solution by measuring the absorbance of the coloured solution at the wavelength of maximum absorption 500 nm. The result was expressed as rhein by reference to a linear calibration graph prepared using a sample of pure rhein (E 1%, 1 cm 500 nm = 330 in N sodium hydroxide. Melting point = 321–322°; Oestele & Tisza (1908) give 321–321·5°).

All experiments were made in triplicate and the results are shown in Figs 1 and 2. At the completion of each experiment the amount of total water-soluble solids

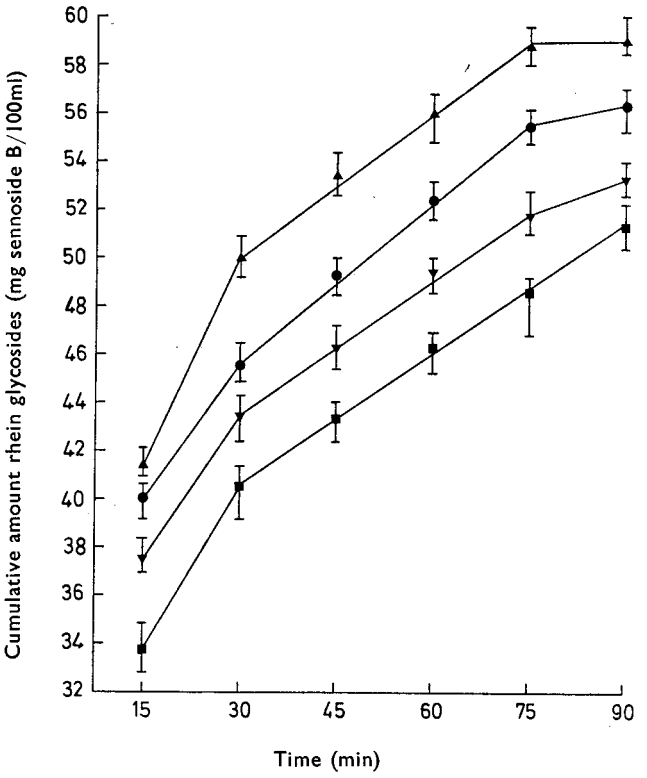

FIG. 1. Effect of ultrasonic energy, controlled heating and ultrasonic energy at room temperature on the extraction of rhein glycosides in aqueous extracts of senna pericarps. ■, Room temperature; ▼, ultrasonic energy at room temperature; ●, controlled heating; ▲, ultrasonic energy. Symbols indicate mean of 3 experiments and vertical lines indicate variation in 3 experiments.

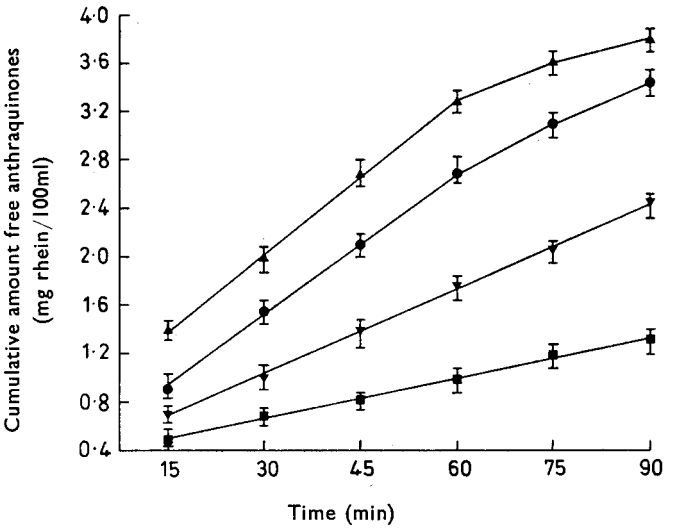

FIG. 2. Effect of ultrasonic energy, controlled heating and ultrasonic energy at room temperature on the extraction of free anthraquinones in aqueous extracts of senna pericarps. ■, Room temperature; ▼, ultrasonic energy at room temperature; ●, controlled heating; ▲, ultrasonic energy. Symbols indicate mean of 3 experiments and vertical lines indicate variation in 3 experiments.

extracted was determined by filtering the solution through a No. 4 Whatman filter paper and drying two 30 ml portions to constant weight at 105°.

Effect of ultrasonic power

The extraction technique was repeated in triplicate by subjecting the pericarp suspension to ultrasonic energy for 15 min using a Soniprobe type 1130* (frequency 20 kHz, probe end diameter $\frac{1}{2}$ inch) adjusted to generator power outputs of 35, 60 and 100 W. The procedure was also carried out at $20 \pm 0{\cdot}5°$ by surrounding the extraction vessel with an ice-salt mixture and the results compared with those obtained using an MSE 60W ultrasonic disintegrator† (frequency 20 kHz, probe end diameter $\frac{3}{8}$ inch).

Effect of ultrasonic energy on pericarp epidermal cells

Portions of epidermal cell tissue were removed from pericarps which had been ultrasonically irradiated for 45 min and the result compared microscopically with samples from non-insonated material. Cell debris in the extraction vessel after 90 min ultrasonic irradiation was similarly examined and the results are shown in Fig. 3.

Effect of size reduction

Whole pericarps (1 g) were suspended in purified water (100 ml) in a stoppered conical flask and the latter left at room temperature for 24 h with constant agitation. The content of rhein glycosides and total water-soluble solids in the filtered extract was determined as described above and the method repeated using pericarps in moderately fine powder.

* Dawe Instruments Limited, London, W.3.
† Measuring and Scientific Equipment Limited, S.W.1.

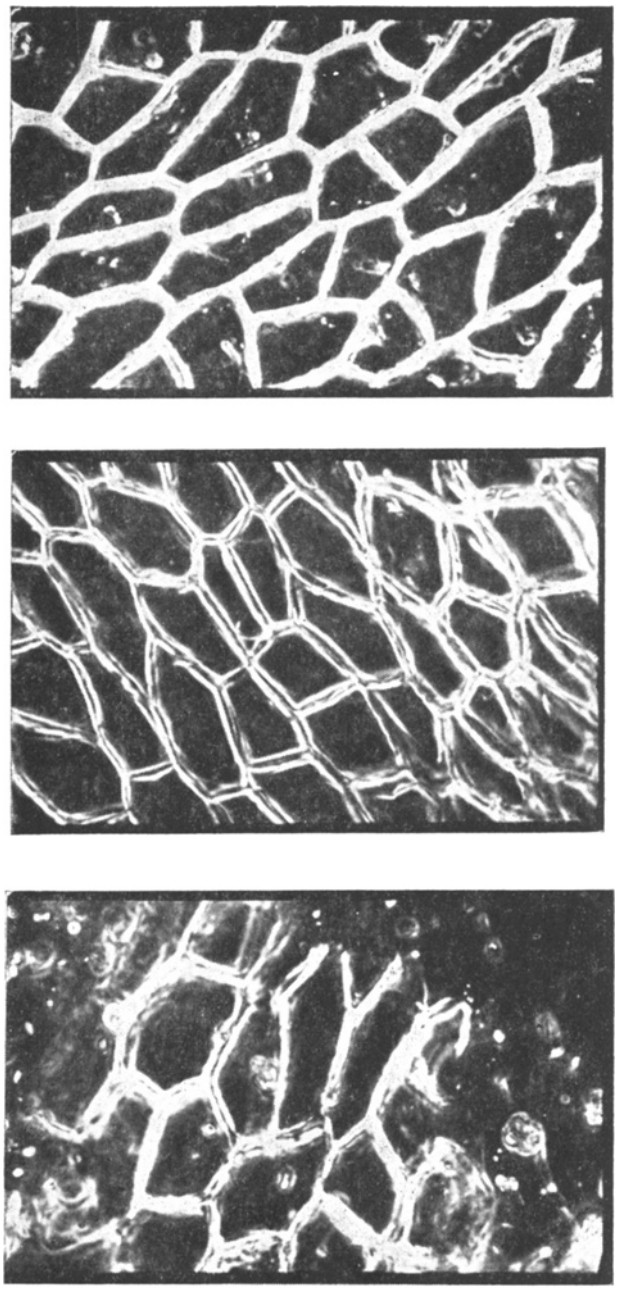

FIG. 3. Effect of 60 W ultrasonic energy on pericarp epidermal cells. (a) Non-insonated pericarp. (b) Pericarp insonated for 45 min. (c) Pericarp insonated for 90 min. Cell debris in extraction vessel.

DISCUSSION

Preliminary experiments showed that ultrasonic irradiation increased the extraction rate of rhein glycosides from whole pericarps but not the total amount extracted.

Over a 90 min period the mean increases over room temperature maceration due

Table 1. *Effect of ultrasonic energy, controlled heating and ultrasonic energy at room temperature (20°C) on the extraction of total water-soluble solids after 90 min in aqueous extracts of senna pericarps*

	Amount of total water-soluble solids in aqueous extracts (mg/100 ml)			
	Ultrasonic energy	Controlled heating	Ultrasonic energy at room temperature	Room temperature
Mean value of 6 determinations	227	219	210	200
Standard deviation	4·9	3·7	4·2	4·2
% increase over room temperature	13·5	9·5	5·0	—

Value of t for ultrasonic energy and controlled heating = 4·84, ultrasonic energy at room temperature and room temperature = 6·32, ultrasonic energy and room temperature = 17·68 ($P' = 0{\cdot}05$ and 10 degrees of freedom, $t = 2{\cdot}23$).

to ultrasonic energy at room temperature, controlled heating and normal ultrasonic extraction were: 6·6%, 13·2% and 20·4% for the rhein glycosides; 71%, 151% and 204% for free anthraquinones; and 5·0%, 9·5% and 13·5% for total water-soluble solids (Table 1). In all instances these results are in the approximate ratio of 1 : 2 : 3. Neither the temperature rise produced by insonation (31·5° over 90 min) nor ultrasonic energy itself produced any obvious chemical degradation of senna extracts as shown by spectrophotometric examination and paper and thin-layer chromatography using 10 solvent systems.

The rate of hydrogen peroxide formation in water insonated using the MSE disintegrator (Morrison & Woodford, 1967) was independent of temperature over the range 20 to 60°. The consistency of the results obtained from successive experiments using the same container and depth of probe immersion indicated that no significant change in cavitation intensity occurred during the extraction of senna pericarps.

The results in Table 2 show that the temperature of the insonated liquid and the yield of extractable constituents increased with ultrasonic power, the proportion of

Table 2. *Effect of ultrasonic power on the extraction of rhein glycosides, free anthraquinones and total water-soluble solids after 15 min in aqueous extracts of senna pericarps*

Ultrasonic power W	Temperature of extract after 15 min	Rhein glycosides (mg sennoside B/100 ml) (a)	Free anthraquinones (mg rhein/100 ml)	Total water-soluble solids (mg/100 ml) (b)	a/b × 10^3
—	20	33·7	0·57	270	125·0
35	20	38·1	0·63	281	135·5
	29	41·1	1·01	301	136·7
60	20	39·5	0·76	293	134·8
	38	43·2	1·46	318	135·8
60*	20	37·5	0·74	285	131·6
	37	41·3	1·40	312	132·4
100	20	45·7	1·03	341	134·1
	67	54·1	1·90	401	135·0

Each value is the mean of 3 experiments. * MSE 60W ultrasonic disintegrator; other values refer to Soniprobe equipment.

active principles in the extract being slightly lower when ultrasonic irradiation was at 20° than when the temperature was uncontrolled. The greatest yield of rhein glycosides was obtained using high power irradiation but although the ratio of sennosides to total extractive increased by 1·04% at 20° using low power ultrasound this was not statistically significant. Little information is available about the effect of ultrasonic power on the yield of active principles but Kubiak (1962) reported that an increase in intensity produced an increase in glycoside yield from frangula bark over a 20 min insonation time.

The ratio of rhein glycosides to total water-soluble solids obtained using the MSE disintegrator was 2·5% lower than that observed for the Soniprobe adjusted to the same power output but this was not statistically significant.

Microscopical examination of epidermal tissue showed that exposure to ultrasonic energy for 45 min produced visible damage to occasional cell walls (Figure 3b) as well as more rapid extraction of mucilage and other cell contents. In addition, where the pericarp had been directly in the path of cavitation streamers, complete removal of small areas of epidermal tissue was noted and after 90 min the extraction vessel contained obvious cell debris (Figure 3c).

The results in Table 3 indicate that cell wall rupture using a high speed mill increased the extraction rate of rhein glycosides from senna pericarps. Cell walls not ruptured during milling did not show the occasional abraded appearance of those exposed to ultrasonic energy but extracts prepared using comminuted or insonated tissue contained significant amounts of colloidal matter absent from normal whole-pericarp macerates. The $E(1\%, 1\text{ cm})$ value at 440 nm for the red solution produced during the rhein glycoside assay is raised when impurities are present (Recommended Method) but the mean value for the ratio E (515 nm)/E (440 nm) obtained was 1·42, irrespective of the presence of colloidal matter.

The results of this study indicate that the specific effect of ultrasound on the extraction rate of senna pericarps is not due to the chemical affects of cavitation. Sennoside determinations using different sieve fractions of comminuted material showed that the rhein glycosides are located in the non-fibrous tissue and damage to epidermal cells, together with facilitated removal of mucilage, may contribute to the increased extraction rate of active and inactive constituents produced by ultrasonic energy.

Table 3. *Effect of size reduction on the aqueous extraction of senna pericarps*

	Whole pericarps			Pericarps in moderately fine powder		
	Rhein glycosides (mg sennoside B/100 ml) (a)	Total water-soluble solids (mg/100 ml) (b)	$a/b \times 10^3$	Rhein glycosides (mg sennoside B/100 ml) (a)	Total water-soluble solids (mg/100 ml) (b)	$a/b \times 10^3$
Mean value of 10 determinations	53·7	327	164·3	59·3	371	159·9
Standard deviation	0·94	2·2	0·11	1·46	9·6	0·16

Value of t for rhein glycosides = 10·16, total water-soluble solids = 14·09, ratio of rhein glycosides to total water-soluble solids = 2·48 ($P' = 0{\cdot}05$ and 18 degrees of freedom, $t = 2{\cdot}10$). Rhein glycoside content of senna pericarps = 63·8 mg sennoside B/g (mean of 5 determinations, standard deviation = 0·58).

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