

CHEMICAL EVALUATION OF *CITRULLUS COLOCYNTHIS*

by

F. W. KHAN & PAZIR GUL*

SUMMARY

Determination of the active constituents and solvent extractives from the Colocynthis showed that the indigenous species was of good quality and can be used in the manufacture of mild mercurous chloride pills.

Yield and chemical characteristics of the oil from the seed of the Indigenous species were compared favourably with those of the exotic species given in the literature. It was observed that the oil, being of semi-drying type, can be used in cosmetics and varnish industries.

Introduction

Citrullus colocynthis grows as a wild weed in the sandy areas of Pakistan as well as in many other countries like India, North Africa, Syria, Cyprus, Egyptian Sudan, Spain and South Western United States (6,15). In some of these countries it is cultivated as medicinal plant (6). The fruit of the *Citrullus colocynthis* resembles a small apple in size and it is therefore known as bitter apple or *colocynth* (15).

The drug is obtained from both wild and cultivated plants. Flowering takes place from May to August. The fruits are collected in the autumn when they begin to turn yellow. They are peeled with a knife and dried in the sun or by artificial heat. The peeled fruits are from 4-7cm. in diameter and show occasional small patches of imperfectly removed rind. The pulp is white or pale-yellowish-white in colour and very light in weight. The seeds, if not removed, number from 200 to 300 in each fruit and form about 70% of the total weight. The official drug, however, consists of the dried pulp containing not more than 5% of the seeds and not more than 2% of the outer sclerenchymatous part of the pericarp (4, 5).

*The authors are Junior Bio-chemist and Technical Assistant respectively at the Pakistan Forest Institute Peshawar.

Literature

The fruits possess therapeutically active constituents which are much more powerful purgative than any of the drug belonging to emodine group (6,12). Walz (1858) and other early workers attributed the purgative action of *Colocynth* to a glycoside called colosynthin. According to Power and Moore (1910) the purgative action is due partly to an amorphous alkaloid and partly to an amorphous resin. The drug also contains the physiologically inactive α -elaterin (2, 15). The *colocynth* extract is employed more often in preparing compound colocynth extract which is used in the manufacture of compound mild mercurous chloride pills (12).

The active constituents of the fruit pulp include, a bitter glycoside, higher alcohol and traces of alkaloid (4,11,13). A glucoside isolated by extracting the crushed defatted fruit with chloroform, was found to exhibit anticancer activities (4,11).

The acid insoluble ash in the fruit is from 1.6% and the light petroleum ether extract is 3%. Alcohol extract is in the range of 20.4-31.8% (15). The mesocarp contains 1.5% sugar calculated as glucose on the fresh mesocarp basis (1). Seeds of the *Colocynth* yield from 13 to 19% yellow oil (5,15), which on refining gave the following characteristics; refractive index at 25°C = 1.4725, specific gravity = 0.9168, acid value 0.1, saponification value = 197 and iodine value (wijis) = 128 (7). The oil contains 8.9% palmitic acid, 5.6% stearic acid, 17.2% oleic acid and 65% linoleic acid (2).

The quality of the *colocynth* may be judged by microscopical examination together with determination of acid insoluble ash and the percentage of extractives yielded to solvents (15).

Material and Method

Colocynths collected from the sandy areas of Mian-Wali were brought to the laboratory, Pulp, rind and seeds were separated and each part was analysed separately for the active constituents.

Separation of Walz's Colocynthin

The pulp was dried and powdered and 1 kg. of it was extracted with alcohol. The extract was evaporated and the residue (140gm) was treated with water. The Water extract thus obtained was treated with neutral lead acetate followed by lead acetate basic and filtered. The filtrate was then freed from lead acetate by passing H_2S . Colocynthin was precipitated by treatment with tannic acid. The precipitate was collected and washed several times with water and was then mixed with lead oxide. This mixture was continuously treated with solvent ether and a yellowish-brown ether extract was collected (3). It yielded shining crystalline substance melting at 148°-150°C.

Separation of Glucoside

One kg. powdered pulp of the *colocynth* was defatted with light petroleum ether. The defatted material was treated with chloroform which gave 45 gm dark brown residue. The residue was extracted with ether which yielded 3.8 gm. yellowish shining crude glucoside having melting point 147°-153°C. This was dissolved in ethanol and then filtered. The filtered solution was evaporated and a residue was mixed with keiselghar and extracted again with ether (sexhlet). This process was repeated several times, until a yellowish crystalline glucoside having a sharp melting point of 158°-160°C was obtained. It gave brown colour with $H_2SO_4(4)$.

Separation of Pectin

Colocynth juice was prepared by extracting it with water and the volume of the extract was reduced to one quarter by evaporating it on a water bath. The pectin was precipitated with 95% alcohol (1,8). The precipitate was collected in Gooch crucible and washed with alcohol thoroughly. It was dried in an oven at 80°-100°C to a constant weight. The yield of pectin was 16% on dry fruit basis.

Determination of Sugar

Total, reducing and non-reducing sugars were determined in the pulp and rind part of the *colocynth* by titration method using pavy's reagents.

Preparation of Extractives

Keeping in view the fact that the quality of the *colocynth* may be judged by the determination of the acid insoluble ash and the percentage of extractives yielded to solvents, extractives of the pulp and rind part of the *colocynth* were made separately with solvents such as alcohol, water, hexane, chloroform and ether.

Chemical Composition of Oil

After separation from the fruit the seeds were cleaned, dried and ground to a fine powder. Oil was extracted (soxhlet) from the seeds with petroleum ether (40°-60°C). The Purification, physico-chemical constants and the estimation of fatty acids of the oil were carried out by the methods given by Jamieson, Jacobs and Rosenthaler (9,10,14).

Results and Discussion

The glucoside (colocynthin) isolated from the Indigenous species was found to have the same melting point as that of Wälz's colocynthin.

Citrullus colocynthis fruits yielded 16% pectin showing that the same are a good source of pectin as well.

Total, reducing and non-reducing sugars, from the pulp and rind part of the *colocynth*, were determined and the results are tabulated in Table I.

TABLE I

S. No.	Part analysed	Total sugars %	Reducing sugars %	Non-reducing sugars %
1.	Pulp	1.64	1.48	0.16
2.	Rind	1.42	1.11	0.31

The total sugars determined (1.4-1.6%) in the indigenous species were comparable with the total sugar content (1.5%) of the exotic species given in the literature (1).

Extractives of the pulp and rind part of the *colocynth* with different solvents were made and the results are tabulated in the Table II.

TABLE II

S. No.	Constituents	Part of the plant analysed		Reported in literature.
		Pulp	Rind	
1.	Total ash	14.75%	12.10%	—
2.	Acid soluble ash	11.60%	10.88%	—
3.	Acid insoluble ash	2.45%	1.22%	1-6.0%
4.	Moisture	9.50%	9.80%	—
5.	Water extract	37.40%	38.60%	—
6.	Alcohol extract	13.81%	12.55%	20.40-31 8%
7.	Chloroform extract	5.58%	3.79%	—
8.	Petroleum ether extract	2.62%	2.17%	3.0%
9.	Solvent ether extract	3.25%	1.60%	—

The contents of acid insoluble ash, ether and alcohol extractives were also found to be comparable with those mentioned for exotic species in the literature.

Determinations of the physico-chemical constants and Individual fatty acids of the oil from the seeds of the indigenous species were also made. The results are presented in the Table III below.

TABLE III

Physico-chemical constants and Individual fatty acids.

S. No.	Physico-chemical constants and fatty acids	Found	Reported in (literature)
1.	Specific gravity at 20°C ..	0.9256	0.9168
2.	Ref. Index at 25°C ..	1.4679	1.4725
3.	Acid value ..	0.90	0.10
4.	Saponification value ..	198.00	197.00
5.	Iodine value ..	126.00	128.00
6.	Stearic and palmitic acid ..	12.80%	8.90 and 5.6%
7.	Oleic acid ..	16.20%	17.20%
8.	Linoleic acid ..	—	65.00%

On comparison of the above data it was found that the high acid value causes poor flavour of the oil from indigenous species.

Conclusion

1. It was concluded, from the study of the acid insoluble ash and the extractives to solvents, that the Indigenous species was of a good quality and its extractives can be used in the manufacture of compound mild mercurous chloride pills.
2. It was also observed that the Indigenous species can be utilized as a source of colocynthin and pectin.
3. The oil was found to be of semi-drying type and can, possibly, be used in the cosmetic and varnish industries.

The above mentioned comparative studies show that the indigenous species, available in large quantities from the sandy areas of Pakistan, can also be exploited for commercial purposes.

REFERENCES

1. Alim Chandani, R. L.; R. C. Bademi; Tumia Katti, (1949): J. Indian Chem. Soc. 26, 515.
2. A. M. Abu-Nasr & W. M. Potts, (1953): J. Am. oil Chem. Soc. 30, 118.
3. Bamford, F; Stewart C. P. (1951): Poisons their isolation and identification J. and A. churchill Ltd. London W-1 pp. 183.
4. EL-Khadin, N, M.M; A; Abdul Rehman, (1953): J. Chem. Soc. 4991.
5. Evers, N. and Smith Wilfred, (1955): The analysis of drugs and chemicals pp. 385. Charles Griffin and Company Ltd. 42-Drury-Lane London W. C-2.
6. Ferguson, N. M., (1956): A Text Book of Pharmacognosy pp. 99-100 The Macmillan Company New York.
7. G. Misra : C. R. Mitra and K. N. Kaul, (1962): "Chemical examination of the citrullus colocynthis fruits and seeds" J. Sci. Ind. Res. Vol. 21 B.
8. Howitz, W., (1960): Official Methods of Analysis 9th Ed. A.O. A.C. P.O. Box. 540 Benjamin Franklin Station Washington 4 D.C.
9. Jamieson, G. S., (1943): Vegetable Fats and Oils 2nd Ed. pp. 38,39. Reinhold Publishing Corporation New York.
10. Jacobs, M. B., (1962): The chemical analysis of food and food Products. D. Van nostrand company London.
11. Lavie, shave, Gattlich and Glotter, (1962): J. O. Chem. 27, 4546.
12. Lyman, Rufus. A. and Joseph Sprowls, (1955): American pharmacy. Text Book Pharmaceutical Principles Processes and Preparations pp. 395-96 J. B. Lippin-cott Co. Philadelphia.
13. Robertson, P. and Haber W. Youngken, (1956): Pharmacognosy, The study of natural drug substances and certain allied products pp. 237-38. 2nd Ed. J. B. Lippin-cott. Co. philadelphia.
14. Rosenthaler, L., (1930): The chemical Investigation of plants, G. Bell and sons Ltd. London.
15. Trease, G. E., (1962): A Text Book of pharmacognosy, pp. 307—331; Bailliere, Tindall and Co. London.