KAMALA DYE AS AN ANTHELMINTIC

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For nearly a century, kamala has enjoyed a reputation as an anthelmintic. But in scientific literature, reports about its efficacy have been conflicting. Semper[1] tested the drug on frogs, tadpoles and worms and found that it was definitely toxic. Caius and Mhaskar[2] found it useless against hook worms, ascarids and whipworms and stated that it could not be recommended as an anthelmintic. Beach and Warren[3] on the other hand found it to be a good teniacide for chicken. One of the possible reasons for the above-mentioned differences appears to be due to the large variation in the quality of the material employed.

The British Pharmacopœia (old editions) and B. P. Codex, 1934, specify kamala by means of its microscopic characters and its ash content. In our examination of a large number of samples obtained from various places in India, we found that they had all the required microscopic characters but are highly variable with regard to their ash content. The best of them agreed with the Pharmacopœial limit of 4–5% ash but in most of them, the ash varied from 15–40%. As a matter of fact, Perkin[4] found an ash content of 52·5% for the sample of kamala which he analysed. As high values as 87·3% have also been reported for some commercial samples.[5] High ash is a definite indication of poor quality which may arise as the result of bad processing in the course of collection, allowing red earth and sand to come in, or due to wilful adulteration with ferruginous earth. The quality of kamala could however be considerably improved by garbling. We find that it is much more efficient to adopt flotation and thereby the ash content could fairly easily be brought down to 5%.

The above result would suggest that the impurity in kamala is mostly of inorganic origin. But it need not always be so. For assessing the quality of kamala more correctly it may be necessary to examine it for the active principles and estimate them. Chemical studies have indicated that the chief crystalline component of kamala is rottlerin. The yield of this substance could therefore give information about the quality of kamala. Though Laube[6] and Oettingen[7] failed to get any crystalline components from the kamala samples analysed by them, in our investigation, all the samples that we examined yielded rottlerin. Those that were purified by flotation and that had the B.P. limit for ash, generally gave a yield of 10%

of rottlerin when extracted in the following manner. One gram of the drug was successively boiled with 15 c.c., 10 c.c., and 10 c.c. of benzene for 5 minutes each time and filtered. The combined filtrate was concentrated to 5 c.c. and allowed to stand for 4 hours, when rottlerin separated out as a red crystalline powder. The yield of rottlerin obtained by this method of extraction could be taken as a rough indication of quality for even the crude samples of kamala. It appears possible that the cases where rottlerin was not obtained could be explained as due to unsuitable methods of extraction because rottlerin is easily susceptible to oxidation and leads to the formation of uncrystallizable substances. But this method of extraction is not suitable for correct quantitative assay.

Recently, a proposal has been made for the chemical assay of kamala by treatment of an ether extract of the drug with baryta, acidifying the alkaline solution and again extracting it with ether. The ether is distilled off and the residue is weighed.[8] This method is analogous to the chemical method of assay suggested for the male fern. As in the case of male fern it is obviously not suitable for kamala too, since rottlerin undergoes decomposition under these conditions and inert resinous matter also dissolves in ether and baryta.

In the absence of a precise chemical method, a biological method of assay seemed to offer promise. In order to test the use of fish for this purpose, experiments have now been conducted employing pure rottlerin and extracts of kamala. The fish used are small ones locally available (*Haplochilus panchax*) and the time taken for the fish to lose balance is adopted as the criterion of toxicity according to the original suggestion of Krishnaswami and Seshadri.[9] Besides rottlerin, its methyl ether has also been tested, since in the case of hydroxy-flavones, the methyl ethers are much more toxic than the original hydroxy compounds. The results obtained are given below. An alcoholic solution (less than 10 c.c) of the particular substance is added to a litre of tap water and thoroughly mixed before introducing the fish (6 in number). In the case of the methyl ether one gram of gelatin was first added to the water in order to keep the substance in clear solution.

No.	Name of the Substance	Concentration m. grams/litre	Turning time	Remarks
1	Rottlerin	15	85 min.	Died very soon after removal to fresh water
2	,,	30	37 ,,	,,
3	,,	50	30 ,,	,,
4	Rottlerin methyl ether	5	Unaffected in 24 hours	Acquired brown colour which faded gradually
5	,,	10	,,	,,
6	,,	30	Found dead overnight	,,

It will be clear from the above results that rottlerin is definitely toxic and is an efficient anthelmintic. It should be noted that the fish used did not recover after the experiment when they were transferred to fresh water but died within a minute after turning upside down. This is experienced only with some of the most potent insecticides. The methyl ether is unexpectedly much weaker. In this respect, rottlerin differs from the hydroxy-flavones.

It is possible that rottlerin, at any rate the quantity isolated, does not account for all the toxicity of kamala. We have, therefore, examined the total benzene extract of kamala before and after the removal of rottlerin and also the subsequent alcoholic extract of the residue left after the benzene extraction. With all these, the effect on the fish is very similar to that of rottlerin, the details of the reactions of the fish being just the same. It, therefore, follows that the other toxic components present are very similar to rottlerin, and that the potency of the samples of kamala could be assessed by comparison with pure rottlerin used as standard. Using this method, the contributions of the various fractions towards the anthelmintic value of kamala could be estimated, taking the toxicity of rottlerin as one as explained below.

One gram of kamala was extracted exhaustively by boiling for 5 minutes each time with 15 c.c., 10 c.c., 10 c.c., and 10 c.c. of benzene and the total extract evaporated to dryness; yield 0·6 gram. This was taken up in 30 c.c. of rectified spirit. 2 c.c. of this solution (40 mg. of extract) when added to one litre of water was found to have the same toxicity as 16·5 mg. of rottlerin in the same volume of water. 4 c.c. of the solution (80 mg.) and 5 c.c. of the solution (100 mg.) were equal to 33 and 41·5 mg. of rottlerin. Thus the benzene extract had 0·4 of the toxic intensity of pure rottlerin. Since 10% of total kamala or $\frac{1}{6}$ of benzene extract can be separated out as rottlerin, the total anthelmintic potency of the benzene extract is $2\frac{1}{2}$ times the contribution made by the isolable rottlerin itself. The non-rottlerin portion of the benzene extract (50% of original kamala) accounts for the remaining $1\frac{1}{2}$ and this has about 0·3 of the toxic intensity of kamala.

The subsequent alcoholic extract is about 15% of the benzene insoluble residue and thus, it is 6% of the original kamala. This extract is also toxic; but 100 mg. of it are only equal to 10 mg. of rottlerin in toxicity. Its potency is thus only 1/10 that of rottlerin and since further this portion is small, it contributes very little to the total anthelmintic value of kamala (about 2·5%).

It could therefore be concluded that though the total anthelmintic value of kamala may not be due to rottlerin alone and may be due to the other components, rottlerin constitutes the most important and powerful toxic component of kamala. Weight for weight the best quality of kamala has less than a third of the toxicity of pure rottlerin.

References

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