Quantifying the bioactivity of plant extracts during screening and bioassay-guided fractionation

Our research group is involved in the screening of plants for antimicrobial and anti-oxidative activity and isolation of compounds responsible for these activities by bioassay-guided fractionation. In some cases, we are following up on ethnoveterinary leads, but we also investigate many taxa of one family, the Combretaceae.

There are three main reasons for doing screening studies i.e. to find new lead compounds for developing pharmaceuticals, to confirm the ethnomedicinal use of plants or to develop phytomedicines for use as herbal medicines. Apparently, little effort has gone into developing low technology techniques to facilitate the use of medicinal plants in rural communities. In many screening studies, activities are reported non-quantitatively. Shale et al (1999) for example used agar diffusion assay for antibacterial activity and classified plants with a 0-0.3 mm, 0.3-0.7 mm and 0.7-1.0 mm zone of inhibition as having low, medium and high activity. Even if data on extracts are expressed in quantitative terms such as antibacterial activity in minimum inhibitory concentration [MIC] or anti-oxidant activity in trolox equivalent it is usually still not possible to compare different plants with the results presented.

What typically happens is that plants are extracted, the extract is dried and made up to a known concentration and bioassay is carried out on this solution. If the solution has a high or low activity, it is automatically inferred that the original plant also has a high or low activity. To compare the plants however, the quantity extracted from the plant should obviously be brought into the equation. If an extractant extracts say 20% of the dry plant material of plant A [i.e. 200 mg extracted from 1g] and the extract has an antioxidant activity of 0.6 trolox equivalent, it has a much higher total antioxidant activity than plant B extracting 2% of the dry mass [i.e. 20 mg extracted from 1g] with a trolox activity of 1.2 trolox equivalent. Looking at a table with the antioxidant activities of the extracts one would have concluded that plant A is twice as active as plant B and would be a good source for bioprospecting. However, the calculated antioxidant activity per gram dry mass for plant A is 12 trolox equivalent.g^{-1} compared to 2.4 trolox equivalent.g^{-1} for plant B.

In the case of MIC values, the lower the value is, the more active the extract is. If plant A above had an MIC value of 0.2 mg.ml^{-1} and plant B had a MIC value of 0.1 mg.ml^{-1} the total activity would be 200 mg/0.1 mg.ml^{-1} i.e. 2000 ml.g^{-1} for plant A and 20 mg/0.1mg.ml^{-1} i.e. 200 ml.g^{-1} for plant B. The units are ml.g^{-1} and indicate the degree to which the active compounds in one gram of plant material can be diluted and still inhibit the growth of the tested microorganism [Eloff, 2000].

The same principle holds when bioactive compounds are isolated by bioassay-guided fractionation. If the total activity is calculated at each step it is easy to determine if there is a loss of biological activity along the way. Even more important this approach makes it easier to discover the presence of synergistic effects.

The situation is equivalent to the terms efficacy and potency used in pharmacology. The potency would be the activity in mg.ml^{-1} of the extract and the efficacy would be the activity of the total plant material in ml.g^{-1}.

The principle can be demonstrated with the following example.

If the crude extract to be fractionated, had a mass of 2800 mg with an MIC of 0.23 mg.ml^{-1} the total activity in the crude would be 12174 ml. After the first fractionation the values in Table 1 were found.

Without calculating the total activity, the researcher may very well have continued with fraction B4 that represents less than 10% of the total activity. From the data it is also clear that there was only a c. 3% loss in total activity and 39 mg in mass during the first step.

Table I Total activity in different fractions after the first fractionation

Fractions	Mass in mg	MIC in mg.ml^{-1}	Total activity in ml	% of total
B1	50	0.1	500	4.2
B2	200	1.0	200	1.7
B3	2500	0.25	10000	84.7
B4	11	0.01	1100	9.3
Total	2761		11800	

One of the difficulties we have had to face in screening and bioassay-guided fractionation, is the incomplete solubility of dried extracts even in the same extractant as used originally. To circumvent this problem we try not to dry extracts. To determine the concentration of the extract for quantification purposes we take a small aliquot, dry it and use the values obtained to calculate the original concentration. Extracts are kept at 3-7^{0}C, not in a deep freeze where precipitation may take place. In members of the Combretaceae extracts retained antibacterial and anti-inflammatory activity for several months [Eloff, Jäger and van Staden 2001].

In our work on isolating antimicrobial compounds from members of the Combretaceae and plants used in ethnoveterinary medicine, we have found evidence of synergistic effects in antibacterial assays. We would never have picked this up without quantifying our data during bioassay-guided fractionation.

Many publications on screening of plants for antibacterial activity are rather trivial. Phytomedicine has taken the correct step in its instructions to authors in considering only manuscripts reporting significant activity of better than 100 μg/ml for publication. The Journal may consider requesting authors to report the total activity in the plant as a whole seeing that it requires so little effort and makes it possible to compare the bioactivity in different plants on a rational basis.

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