E2-04:Effect of prolonged boiling on the bioactivity and alkaloid content of *Adhathoda vasica* root bark extract. Implications for ayurvedic drugs manufacture

A M Abeysekera[1], A Bamunuarachchi[1], K T D de Silva[1],
A C S Menike[1], A J J van den Berg[2], R P Labadie[2]
(*[1]Univ. of Sri Jayewardenepura, [2]Univ. of Utrecht*)

The preparation of water extracts of plant material in the manufacture of ayurvedic drugs is traditionally carried out by adding a specified quantity of water and boiling down to a final volume in the ratio of 4:1 or 8:1 (specified). In carrying out this procedure on an industrial scale, plant materials are often boiled for very long periods (8-10 days) - much longer than if the operation is carried out on a domestic scale - for which the traditional procedure has been prescribed. The effect of prolonged boiling on the alkaloid content and immunomodulatory activity of an *Adhathoda vasica* root bark extract, which is used to prepare 2 popular ayurvedic drugs, *vasaka peniya* and *vasakarishtaya* was investigated.

A.vasica root bark (3.12 kg) was heated under reflux with water (2 1) for 9 days, 8 h per day. A sample (10 ml) was withdrawn at the end of each day for analysis. The concentrations of vasicine, vasicinone and vasicinolone were determined by HPLC (μ-Bondapack C18/methanol-water 70:30 containing 0.5% PIC B6). Standard curves were drawn with the pure alkaloids isolated from the plant.

IC_{50} values for the inhibition of the classical (CP) and alternative (AP) pathway of complement activation were determined by the appropriate treatment of lysis data obtained by incubating human serum with sensitized sheep erythrocytes (CP) or rabbit erythrocytes (AP), in the appropriate buffer solutions, in the presence and absence of the extract.

Linear calibration curves passing through the origin were obtained for vasicine ($r=0.85$), vasicinone)$r=0.89$), and vasicinolne ($r=0.93$). The vasicine, vasicinone and vasicinolone contents of the extract were 2.184, 0.259 and 0.068 mg ml^{-1} respectively at the end of the first day. These had risen gradually to 2.401, 0.311 and 0.089 mg ml^{-1} respectively by the end of the 9th day. Thus increasing the boiling time 9 fold increased the levels of bioactive alkaloids only by 9.9% (vasicine), 20% (vasicinone) and 31% (vasicinolone).

The IC_{50} values for CP and AP were 1.2 and 144 $\mu l\ ml^{-1}$ respectively at the end of the first day. The value for CP gradually rose to 5.3 by the end of the 9th day, while the value for AP fluctuated between 155 and 144. Thus CP anticomplementary activity decreased by more than a factor of 4, while the AP anticomplementary activity appeared to be unaffected during the 9 days of boiling.

If the traditional procedure recommended for the preparation of water extracts in ayurveda is interpreted to be a convenient way to control the time of extraction, then present day industrial practice is a violation of the traditional preparation method.

Results show clearly that increased energy expenditure incurred in prolonged boiling is not commensurate with the increased extraction of bioactive compounds in *Adhathoda vasica* extracts. Results also indicate that there is a loss of certain biological activities which may be therapeutically important.

These results are of medicinal and economic importance. Considerable saving in energy and time could be achieved by boiling under reflux for a fixed time. Such a procedure would also help to obtain a standard product.