

Study of the Pretreatment (Shodhana) of Roots of *Plumbago indica* L. in Ayurveda

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Plumbago indica L. (Plumbaginaceae) is a medicinal herb, credited with vast number of potential therapeutic properties which is heavily used in traditional and ayurvedic medicinal systems in Sri Lanka and India. Naphthoquinones are the major secondary metabolites in the roots, of which plumbagin, a volatile compound is predominant. Ayurveda formulations are incorporated with air-dried roots of *P.indica* L. upon subjecting to a pretreatment with lime water and this pretreatment is called "Shodhana". Although traditional practitioners do not have a clear picture to explain why this type of pretreatment is done, they believe it reduces toxicity associated with plumbagin. Here, we report a preliminary attempt to give a scientific basis for the pretreatment by using UV/Vis spectrophotometric and chromatographic methods.

Shodhana process resulted a deep maroon colour extract substantiating plumbagin gives a red color in alkaline pH. After recrystallization using hexane, plumbagin was obtained as orange needles and melting point of 76-77 °C was in accordance with literature. In GC/MS studies, the gas chromatogram showed a single peak and corresponding mass spectrum a molecular ion at m/z 188. The IR and UV results were also in accordance with what is published and confirmed the purity of isolated plumbagin. In the study of pretreatment, three hexane extracts (E₁ – hexane extract of fresh roots upon subjecting to pretreatment, E₂ – hexane extract of used roots after pretreatment, and E₃ – hexane extract of fresh roots without subjecting to pretreatment) were subjected to TLC against isolated plumbagin which was used as the working standard using 9:1, benzene: hexane solvent system. According to the results, E₃ showed a very intense spot (S₁) with R_f - 0.63 that correspond to the plumbagin working standard and six other spots of which two were very intense (S₂ and S₃) while others were of low intensity. In the case of E₁, the S₁ spot was observed with low intensity, S₂ spot was not observed at all while the other spots were in low intensities of which one was an extra spot (S₄). When considering E₂, the S₁, S₂ and S₃ spots were observed intensively (not as intense as in E₃)

while the others in low intensities. All these collectively substantiate the fact that although the pretreatment process does not cause much change in the phytochemical composition of the roots, it causes a reduction in the amount of plumbagin along with the other compounds but not completely. By employing optimized conditions and using the calibration curve, which was developed, by using isolated plumbagin as the working standard, the fresh root sample quantified 8.7±0.1 mg/g of plumbagin, which was lowered by 19.4% upon subjecting to pretreatment. Likewise commercial sample quantified 0.55±0.05 mg/g of plumbagin. There is a large difference in amount of plumbagin between fresh and commercial root samples. This may be due to plumbagin being eliminated during the drying process that commercial samples under go before being marketed. According to anecdotal evidences and published reports, the pretreatment is done to reduce toxicity associated with plumbagin. However, ayurveda formulations are incorporated with air-dried roots (commercial samples) not with fresh roots. If pretreatment is done only to reduce toxicity associated with plumbagin, drying process may be sufficient to reduce plumbagin and it may be possible to exclude the pretreatment in ayurvedic preparations. However, to better understand the changes in phytochemical composition of roots of *P.indica* during drying, a long-term qualitative and quantitative study is under way.

References

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