DOI :10.31357/fapsmph.2011.00549

Analysis and standardization of an Ayurvedic medicinal oil ('Pinda' oil) and the measurement of partition coefficients of some of its constituents

By

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Thesis submitted to the University of Sri Jayewardenepura for the award of the Degree of Doctor of Philosophy in Chemistry on 31st March 2011

DECLARATION

The work described in this thesis was carried out by me under the supervision of Prof. A.M. Abeysekera (Department of Chemistry, University of Sri Jayewardenepura) and Prof. G.M.K.B. Gunaherath (Department of Chemistry, The Open University of Sri Lanka) and a report on this has not been submitted in whole or part to any university or any other institution for another Degree/Diploma.

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Chandani Ranasinghe

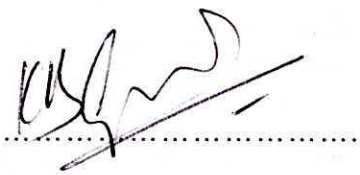
DECLARATION

We certify that the above statement made by the candidate is true and that this thesis is suitable for submission to the University for the purpose of evaluation.

Supervisors:

Prof. A.M. Abeysekera

Date: 19/11/2012



Prof. G.M.K.B. Gunaherath

Date: 19/11/2012

I certify that the candidate has incorporated all corrections, amendments and additions recommended by the examiners.

.....A M Abeysekera..... .....22/12/2012.....

Prof. A. M. Abeysekera Date

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ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to Professor A.M. Abeysekera and Professor G.M.K.B. Gunaherath for the opportunity given to me to carry out this work under their careful supervision. I owe them very much for their guidance, constant support and valuable advice through extensive discussions. Their constructive criticism and comments during the writing up of the thesis had been of immense help.

My sincere thanks are due to Professor U.G. Chandrika, for providing me with the HPLC facility and teaching me how to operate the instrument. She extended her helping hand voluntarily at all times and provided me good company and lots of good ideas.

I appreciate the assistance given by Dr. Ranjala Ratnayake to obtain spectroscopic data and for the suggestions made to solve problems regarding HPLC. Her quick supply of polyamide enabled me carry out my research work uninterrupted.

The assistance given by Mr. Chitraka Wickramarachchi in statistical analysis of the data is gratefully acknowledged.

I wish to acknowledge the technical support given to me by Messers. Siripala Senadeera, Sagara Dias and Sisira Weerasinghe of the Department of Chemistry, University of Sri Jayewardenepura.

I gratefully acknowledge National Science Foundation for the financial support and Link Natural Products Limited for the supply of authenticated raw materials, 'Pinda' oil samples and for permitting me to study the industrial manufacturing process in their factory.

It is a pleasure to thank all my colleagues at The Open University of Sri Lanka, who supported me in numerous ways, with a special mention of Professor Sukumal Wimalasena who helped me in the final stages of preparing the thesis.

Finally I would like to thank my family for their understanding, patience and for being very supportive throughout the course of this study.

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ABSTRACT

'Pinda' oil is a potent and widely used Ayurvedic medicinal oil for the treatment of inflammations and a variety of dermatological conditions such as eczema, itchy skin, cracked skin and red skin. In the manufacture of 'Pinda' oil, the constituents of an aqueous extract of *Rubia cordifolia*, *Glycyrrhiza glabra*, and *Cryptolepis buchanani* are incorporated into sesame oil.

With the commercialization of Ayurvedic drug manufacture, the need for quality control and standardization of drugs has arisen to ensure the quality, potency and the efficacy of these drugs, for the benefit of the consumer.

Analysis and standardization of 'Pinda' oil was a challenging task due to the difficulty in obtaining a suitable extract of secondary plant metabolites incorporated into the oil devoid of fatty matter.

'Pinda' oil was subjected to liquid-liquid partitioning and TLC profiles ('fingerprints') of the fractions thus obtained were developed. The secondary plant metabolites incorporated in to the oil were identified with the help of the major compounds isolated from the three plants used to make 'Pinda' oil. The introduction to this thesis reviews the chemistry and biological activities of these three plants and a compilation of natural products isolated from them is included in Appendix 2.

The five anthraquinones pseudopurpurin, alizarin, purpurin, xanthopurpurin and rubiadin the triterpenoids β-sitosterol, lupeol acetate and α-amyrin acetate, the flavonoids liquiritigenin and isoliquiritigenin and the simple phenolic scopoletin were identified as the major constituents incorporated into 'Pinda' oil.

A phenolic extract comprising of the five anthraquinones was obtained by solid phase extraction of 'Pinda' oil using polyamide as the sorbent. Fats were removed by eluting with *iso*-octane. A RP-HPLC method was developed to quantify the anthraquinones present in this phenolic extract. The chromatograms were obtained by isocratic elution of the phenolic extract using acetonitrile : 1% formic acid in water (65:35) as the mobile phase. Peaks corresponding to the four anthraquinones alizarin, purpurin, xanthopurpurin and rubiadin displayed the required purity levels for quantification.

The precision and the accuracy of the above method were found to lie within the acceptable ranges. Therefore this method was considered a good method for standardizing different market samples of 'Pinda' oil in terms of the anthraquinones present in *Rubia cordifolia.* Analysis showed a wide variability among the samples indicating the need for standardization and quality control of the drug.

The industrial manufacturing process was studied in relation to the rates of incorporation of the four anthraquinones of concern into the oil. Xanthopupurin showed a very slow incorporation into oil in the initial stage of heating while all four anthraquinones reached their concentration maxima by 46 hours of heating. The experiments done by direct extraction of raw material with oil showed lower concentration levels of anthraquinones of concern in the oil thus validated the need for the aqueous extraction step in the traditional preparation process of manufacture.

Measurement of partition coefficients (P) of the most abundant constituents of plant materials were done to study whether there was a correlation between the log P values and concentrations of compounds in the oil. No correlation could be could be observed.