

**Evaluation of *Centella asiatica* morphotypes for high yielding
sources of Asiaticoside and optimizing conditions for higher
yields of Asiaticoside**

By

Thanthiriwaththage Dona Chathurika Kalpanie Dissanayake

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The work described in this thesis was carried out by me under the supervision of Prof. A. M. Abeysekera, Prof. (Mrs.) N. Salim, Prof. (Mrs.) U. G. Chandrika , Dr. (Mrs.) C. Padumadasa and a report on this has not been submitted in whole or in part to any university or any other institution for another Degree/Diploma

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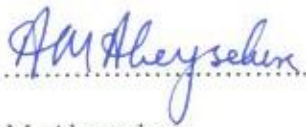
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Certification of approval

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



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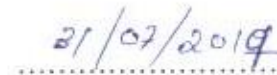


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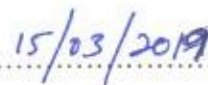
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We certify that all the corrections, additions and amendments have been done in accordance with the comments and suggestions made by the members of the viva – voice examination.



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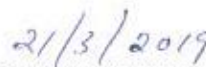


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LIST OF ABBRIVIATIONS

| | | |
|------|---|--|
| ASTC | - | asiaticoside |
| HPLC | - | high performance liquid chromatography |
| TLC | - | thin layer chromatography |
| LDPE | - | low density poly ethylene |
| DoA | - | department of Agriculture – Sri Lanka |
| LAI | - | leaf area index |

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ACKNOWLEDGMENTS

I sincerely thank my supervisor, Prof. A. M. Abeysekera, Emeritus Professor at the Department of Chemistry, University of Sri Jayewardenepura for his encouragement, invaluable guidance and coordinating this project with Link Natural Products, Pvt. Ltd.

I sincerely thank my supervisor, Prof.(Mrs.) N. Salim, Senior Professor at the Department of Botany, University of Sri Jayewardenepura for her encouragement, invaluable guidance and granting the opportunity to carry out the research in the botanical garden and the greenhouse of the department of botany, as the Head of the Department.

I would like to express my sincere thanks to my supervisor, Prof.(Mrs.) U. G. Chandrika, Professor at the Department of Biochemistry, University of Sri Jayewardenepura for her support, guidance and giving the opportunity to use HPLC instrument for the quantitative analysis.

I would like to thank my supervisor, Dr.(Mrs.) C. Padumadasa, Senior Lecturer at the Department of Chemistry, University of Sri Jayewardenepura, for her assistance and guidance.

I would like to express my sincere gratitude to Prof. Subasinghe, Dean, Faculty of Agriculture, University of Ruhuna for guiding me in the agricultural component of the research and designing the field experiments.

I would like to thank Mr. Kumarasinghe, lecturer at Faculty of Agriculture, University of Ruhuna for performing the statistical analysis of the field experiments.

I would like to thank Prof. S.I. Samarasinghe the former head of the Department of Chemistry, University of Sri Jayewardenepura and the current Prof. P. M. Jayaweera head at the Department of Chemistry, University of Sri Jayewardenepura for giving me the opportunity to carry out the research in Post Graduate research laboratory of the Department of Chemistry, University of Sri Jayewardenepura.

A special word of thanks is to Link Natural Products, Pvt. Ltd. for providing the financial support for the project.

I would like to express my sincere gratitude to Mr. Tennakoon, Manager, Division of Research and Development, Link Natural Products, Pvt. Ltd. for his invaluable guidance, support and providing the standard of asiaticoside.

I would like to thank Mr. Buddhisha, manager, Division of Agriculture, Link Natural Products, Pvt. Ltd. for providing the space and support to do field experiments.

I wish to thank all the academic and non – academic staff members of the Department of Chemistry, University of Sri Jayewardenepura and Department of Botany, University of Sri Jayewardenepura, for their cooperation.

I wish to thank all the staff members of the Division of Research and Development and the Division of Agriculture of Link Natural Products, Pvt. Ltd. for their support.

I would like to thank the staff members of the Faculty of Graduate studies for the kind co – operation given to me at all the times needed.

Finally I would sincerely thank my husband Mr. D. H. M. M. Dhananjaya for his encouragement and cooperation.

ABSTRACT

Centella asiatica (L) Urban, a herb native to South East Asian and African regions is used in many traditional medicinal systems. The bioactivities related to many of the traditional uses of the plant have been confirmed by modern scientific studies. Out of the most active major compounds in *C. asiatica*, asiaticoside has shown the best wound healing, anti-aging and scar removal properties, which has lead to the compound being used in cosmetic products.

Different morphotypes of *C. asiatica* have been identified from Sri Lanka as well as other countries such as India, South Africa and Madagascar. It has been reported that there is a variation in the asiaticoside content among the different morphotypes in India, South Africa and Madagascar. The variation in vitamin and mineral contents and susceptibility to root knot nematode infestation has been reported among Sri Lankan morphotypes. Identifying the morphotypes with high asiaticoside content and the agronomic conditions for their cultivation for optimum yields of asiaticoside is of industrial importance. This thesis reports the work carried out to find answers to these questions.

A convenient method of extraction and quantification of asiaticoside from *C. asiatica* plant material was developed and validated. Using the developed method the asiaticoside content of five different morphotypes were analyzed.

Defatting the dried plant material with chloroform and extracting with methanol provided a quantitative extract of asiaticoside, suitable for HPLC analysis. Reverse phase HPLC in isocratic mobile phase acetonitrile: water ratio at 30:70, 1 ml/min at 26 °C was a convenient method of quantification of asiaticoside in extracts. The method developed was precise (RSD = 1.7%) and accurate (99.8% recovery at an addition rate of 20 %), and convenient, to analyze a large number of field samples.

Out of the morphotypes tested, the morphotype, G 3 showed a significantly higher asiaticoside yield consistently (2.70 ± 0.25 in pot condition and 1.12 ± 0.45 and 1.60 ± 0.16 in field condition). Therefore the effect of shade level, fertilizer treatment, harvesting frequency and plant spacing on biomass and asiaticoside yield in morphotype G3 were evaluated in factorially designed field trials,

The biomass and asiaticoside yield of G3 was higher in the shade level 30% when compared to 50% shade level. There was no significant difference in asiaticoside and biomass yields between the organic and inorganic fertilizer treatments recommended by the Department of Agriculture (DoA), Sri Lanka. a 1:1 mixture of these two resulted in lowering in biomass and asiaticoside yield. There was no significant difference in asiaticoside yield among the four different harvesting periods or among the five different spacing treatments tested. However difference in biomass yield was observed.