VALUE ADDITION AND STANDARDIZATION OF PLANT PRODUCTS FOR RURAL INDUSTRIALIZATION (*STEVIA REBAUDIANA* AND *BOERHAAVIA DIFFUSA*)

by

RICHA SHRIVASTAVA

Centre for Rural Development and Technology

Submitted in fulfilment of the requirement of the degree of Doctor of Philosophy

to the

INDIAN INSTITUTE OF TECHNOLOGY, DELHI
HAUZ KHAS, NEW DELHI
FEBRUARY 2008

Medicinal Plants - Production ... Kenya

CERTIFICATE

This is to certify that the thesis entitled, "VALUE ADDITION AND STANDARDIZATION OF PLANT PRODUCTS FOR RURAL INDUSTRIALIZATION (*Stevia rebaudiana* and *Boerhaavia diffusa*)", being submitted by Ms. Richa Shrivastava to Indian Institute of Technology, Delhi for the award of Doctor of Philosophy is a record of bonafied research work carried out by her under our guidance and supervision in conformity with the rules and regulations of Indian Institute of Technology, Delhi. The research report and results presented in this thesis have not been submitted in part or full to any other university for the award of any degree or diploma.

(PROF. R. C. MAHESHWARI)
Supervisor
Ex-Professor
CRDT, IIT Delhi
New Delhi 110016
Principal, HCST
Farah, Mathura

(Dr. S. N. NAIK)
Supervisor
Associate Professor
CRDT, IIT Delhi
New Delhi 110016

Acknowledgement

I am extremely grateful to the almighty for giving me this opportunity and the strength for bringing it to completion.

I gratefully acknowledge the guidance of my supervisors Prof. R.C. Maheshwari and Dr. S. N. Naik for the selection of the research topic and guiding me for its realization into a thesis. I would like to give a special thanks to Mrs. Maheshwari for her encouragement, love and support.

I am thankful to Dr. Bedi and Dr. Suri, RRL Jammu for contributing valuable information and providing the facility for LC-MS. I wish to thank Dept. of Science and Technology for financial support. I wish to express my deepest thanks to Mr. Sanjay Shrivastava, Dr. V.K. Singh, Dr. M.R. Uniyal, Maharishi Ayurveda for Instrumentation facility and the help they provided during the research work.

I am grateful to Prof. Santosh Satya, Dr. Satyawati Sharma, Dr. V. K. Vijay and Prof. Rajendra Prasad, laboratory and office staff of the Centre for providing lab and other facilities for my research work.

I would like to thank all my friends Ghazala, Lekha, Neetu, Madhu, Giriraj, Manish and Parag for their support and encouragement. I would especially like to thank Archana and Vishal for their valuable suggestions and help.

The support and encouragement from my husband, Mr. Prashant S. Negi has been invaluable to me. Also the blessings, love, patience and support of my family, especially my Grandfather and Grandmother during the course of my research work have been immense. I am especially thankful to my parents and parents in law.

Richa Shrivastava

Abstract

Rural economy is mostly dependent on agriculture. But agriculture of usual cereal crops will no longer be able to give high returns to the farmers as these crops come under surplus production with their prices becoming lower or more or less stable. Therefore, to facilitate the development of rural areas and improve rural economy it is recommended to modify the cropping pattern, wholly or in part coupled with industrialization. Cultivation of high value commercial crops along with their agro-processing at the rural level itself will help in sustainable development of the rural areas. Value added products will give much better revenue than just the raw material.

Standardization is an important aspect in terms of quality of the product. Proper standardization can increase the value of a manufactured product. Lack of this aspect in the traditional medicine industry in India is the cause of limited exports of herbal and Ayurvedic medicines.

Considering these factors two plants Stevia rebaudiana and Boerhaavia diffusa were selected for the present research work. Both these are high value plants that have increasing demand in the market.

Stevia rebaudiana is a plant containing natural low calorie sweet compounds in its leaves. This plant is a native of Paraguay and has been introduced in India in the last decade. Its cultivation is on the rise but it is mostly sold or exported in the raw material form of dried leaves in India. A simple process for the extraction and purification of the main sweet compound, stevioside from the leaves of Stevia has been developed in the present study. It involves extraction of the Stevia leaf powder by boiling water followed by a four step purification process. The extraction parameters

were optimized. The purification was carried out by adsorption process, followed by ion exchange resins and finally by ultrafiltration membrane. The basic objective of purification was to lower the quantity of compounds other than stevioside in the extract and to remove the coloured compounds such as tannins from the extract. The quality of the extract was checked by analysing three main parameters; they were quantity of stevioside in the extract, the colour absorbance of the extract and the total solids present in the extract.

The final extract was concentrated by using a reverse osmosis membrane to remove extra water and lower molecular weight impurities from the extract. After which the extract was concentrated under vacuum and dried in a vacuum oven to yield fine off-white coloured crystals. The purity of the stevioside obtained was analysed and the yield was calculated.

A small scale project for rural entrepreneurs has been proposed based on the process developed in the current research work. It could be set up in the rural areas where cultivation of Stevia is being done on large scale. Also places where Stevia is being cultivated in India and where it can be cultivated have been listed out. The cost economics of Stevia cultivation has also been worked out. Stevia cultivation along with its value added products such as liquid extracts or stevioside powder can be a beneficial project for rural areas as the demand for natural sweeteners is rising in the international as well as domestic market. Diabetes is a major problem worldwide with India being a country with highest number of diabetics; a low calorie natural sugar substitute will be a major commodity in times to come.

Boerhaavia diffusa is one of the most important Ayurvedic plant for liver and kidney disorders. It is also found to be useful in diabetes owing to its anti-inflammatory and diuretic properties. The reason for selecting this plant was its wide

use in traditional medicine but lack of standardization. Its whole plants and root both are recommended for medicinal purpose but generally the roots are used. The roots are rich in alkaloids and phytoecdysones. The roots were extracted in a Soxhlet apparatus with 95 % ethanol and partitioned with organic solvents to obtain the alkaloid fraction. Characterization of this fraction was carried out by using TLC and LC-MS. The quantity of main alkaloid Punarnavine present in the roots of Boerhaavia diffusa can be an important parameter of quality of the raw material.

The study of β-ecdysone was also done in Boerhaavia diffusa root extract by TLC and HPTLC. Its quantity was estimated in the roots and in samples of Ayurvedic medicines containing Boerhaavia diffusa as an ingredient. β-ecdysone was recommended as biological marker for this plant owing to its important pharmacological properties and commercial availability. The technique for its estimation can be used as an important tool for the standardization of the plant and its products.

The cultivation of this medicinal plant is highly recommended as its demand in the market is high and it is collected from the wild.

Boerhaavia diffusa was taken as an example from the numerous medicinal plants in India that require systematic cultivation and standardization. With growing awareness about the traditional medicines and an increasing demand for them, these plants can play a very important role in rural development.

CONTENTS

	Page No.
Acknowledgment	i
Abstract	iii
List of Figures	ix
List of Tables	x
List of Plates	xi
Notations	xii
Chapter 1: Introduction	**1**
1.1 Background	1
1.2 Rural Industrialization through *Stevia rebaudiana* and *Boerhaavia diffusa*	9
1.3 Value Addition	11
1.4 Standardization of plant products	13
1.5 Significance of Stevia over other plants having sweet compounds	14
1.6 Importance of *Boerhaavia diffusa* for rural development	16
1.7 Standardization of Ayurvedic Medicines	16
1.8 Formulations of selected for study	18
Chapter 2: Review of literature	**20**
2.1 *Stevia rebaudiana*	20
2.1.1 General Description	20
2.1.2 Place of origin and its introduction in India	20
2.1.3 Cultivation of Stevia in India	23
2.1.4 Chemical Constituents	28
2.1.5 Extraction and purification of stevioside	29
2.1.6 Analysis of stevioside	36
2.1.7 Pharmacological studies	38
2.2 *Boerhaavia diffusa*	40
2.2.1 General description	40
2.2.2 Chemical constituents	42
2.2.2.1 β-ecdysone	44
2.2.2.2 Analysis of β-ecdysone	46
2.2.2.3 Punarnavine	46
2.2.3 *Boerhaavia diffusa* in Ayurveda	47
2.2.4 Ethnobotanical uses of *Boerhaavia diffusa*	52
2.2.5 Pharmacological studies	53
2.3 Scope & Objectives of the present study	57
2.4 Methodology	60
2.4.1 *Stevia rebaudiana*	60
2.4.2 *Boerhaavia diffusa*	60

Chapter 3: Materials and Methods **61**
3.1 *Stevia rebaudiana*: Extraction and purification of stevioside 61
3.1.1 Selection of Plant material 61
3.1.2 Preparation and screening of Plant material 62
3.1.3 Analytical methods 63
3.1.3.1 Preparation of samples 64
3.1.3.2 Thin Layer Chromatography 65
3.1.3.3 High Performance Liquid Chromatography 66
3.1.3.4 UV-Visible Spectrophotometry 66
3.1.4 Extraction of Stevia leaves 67
3.1.4.1 Optimization of extraction process 67
3.1.5 Purification of stevioside 70
3.1.5.1 Clarification of Stevia leaf extract by adsorption process 70
3.1.5.2 Ion Exchange 72
3.1.5.2.1 Specification of Ion exchange resins used 73
3.1.5.2.2 Purification of Stevia leaf extract by Ion Exchange resins 75
3.1.5.2.3 Regeneration of resins 76
3.1.5.2.4 Storage of resins 76
3.1.5.3 Ultrafiltration 76
3.1.5.3.1 Membrane selection 77
3.1.5.3.2 Membrane specifications 77
3.1.5.3.3 Ultrafiltration of Stevia leaf extract 78
3.1.6 Concentration of the purified extract 82
3.1.6.1 Membrane selection 82
3.1.6.2 Specifications of the RO membrane 83
3.1.6.3 RO concentration of purified extract 84
3.1.7 Final concentration of purified extract 85

3.2 *Boerhaavia diffusa*: Characterization and estimation of active compounds 87
3.2.1 Plant material 87
3.2.2 Preparation of Plant material 87
3.2.3 Extraction of alkaloid fraction 88
3.2.4 Characterization of alkaloid fraction 91

3.3 Identification and estimation of β-ecdysone in roots and formulations of *Boerhaavia diffusa* 94
3.3.1 Sample preparation 94
3.3.2 TLC of *Boerhaavia diffusa* root and formulation extracts for identification of β-ecdysone 95
3.3.3 HPTLC of *Boerhaavia diffusa* root and formulations for quantification of β-ecdysone 96

Chapter 4: Results and Discussion **98**
4.1 *Stevia rebaudiana*: Extraction and purification stevioside 98
4.1.1 Preparation of Plant material 99
4.1.2 HPLC analysis for screening of the raw material 99
4.1.3 Extraction of stevioside 100
4.1.3.1 Optimization of stevioside extraction 100
4.1.4 Purification 108
4.1.4.1 Clarification by adsorption process 108

4.1.4.2 Ion exchange 114
4.1.4.3 Ultrafiltration 115
4.1.5 Concentration of purified extract 120
4.1.6 Final Product 122
4.1.7 Yield of stevioside 122
4.1.8 TLC and HPLC analysis 123

4.2 Extraction and characterization of alkaloid from *Boerhaavia diffusa* roots 130
4.2.1 Characterization of *Boerhaavia diffusa* alkaloid extract by chemical tests and TLC 130
4.2.2 Characterization of *Boerhaavia diffusa* alkaloid extract by LC-MS 132

4.3 Identification and estimation of active compound in roots & formulations of *Boerhaavia diffusa* 139
4.3.1 Identification of β-ecdysone 139
4.3.2 Estimation of β-ecdysone 142

Chapter 5: Relevance of the present study for Rural Industrialization 145

Chapter 6: Conclusion and Future scope 161

References 167

Biodata 189